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THE UNIVERSITY OF ALBERTA

AN INVESTIGATION INTO THE MECHANISM OF RESPIRATORY WHEEZE IN CARDIAC ASTHMA

BY

© KOON KANG TEO

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

MEDICAL SCIENCES (MEDICINE)

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled An Investigation into the Mechanism of Respiratory Wheeze in Cardiac Asthma, submitted by Koon-Kang Teo in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Medical Sciences.

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Supervisor

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.....

.....

.....

Date: October 5, 1987.

DEDICATION

TO

MY PARENTS FOR THEIR ENCOURAGEMENT AND UNDERSTANDING,

AND

MY WIFE PEARLIE FOR HER SUPPORT AND PATIENCE.

ABSTRACT

This study was designed to examine a possible mechanism by which respiratory wheeze develops in pulmonary congestion as found in cardiac asthma.

Experiments were performed on thoracotomised dogs anaesthetized with α-chloralose. The influence of sustained pulmonary venous congestion, produced by partially obstructing the mitral valve and elevating the left atrial pressure (LAP) for 15 min, on the vagal sensory receptors of the airways was studied.

Fourteen out of 15 slowly adapting (SAR), all 11 rapidly adapting (RAR) and all 9 bronchial C-fibre receptors showed significant sustained increases in activity (+18%, 153% and +50% respectively, $p<0.005$) when LAP was elevated by 9.4 ± 0.2 mm Hg. Six out of 9 pulmonary C-fibre receptors examined also increased their activity, but the change in this group was not statistically significant. Also, all 7 SAR, 5 RAR and 5 bronchial C-fibre receptors, but not 5 pulmonary C-fibre receptors, examined showed significant graded increases in activity when LAP was increased in steps of 5 mm Hg each up to 15 mm Hg..

Sustained pulmonary venous congestion (LAP $+10.7 \pm 0.2$ mm Hg) increased the tension in an upper tracheal segment with an intact motor innervation by 6.5 ± 0.6 g, from a baseline of 91.8 ± 2.1 g ($n=53$ in 17 animals, $p<0.001$). This reponse was abolished reversibly by cooling the cervical vagi to 8°C. It was also abolished by vagotomy, administration of atropine and cutting the superior laryngeal nerves. Stimulation of atrial receptors without associated

increases in LAP did not cause a change in tracheal tension.

The results show that (1) at a modest level of pulmonary venous congestion, the RAR were stimulated maximally, (2) the increase in tracheal tension is a reflex phenomenon with the afferent link in the myelinated fibres in the vagi and the efferent link in the superior laryngeal nerves. The RAR are the afferents most likely involved in the reflex.

It is hypothesised that an increase in the fluid fluxes in the extravascular interstitial spaces of the lungs and airways produced by pulmonary congestion could result in the excitation of the RAR. Stimulation of the RAR reflexly caused an increase in tone of the airway smooth muscle. This could be the mechanism of the respiratory wheeze seen in cardiac asthma.

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CHAPTER 1

INTRODUCTION

The clinical syndrome of cardiac asthma, seen during the acute pulmonary congestion that follows left ventricular dysfunction, is well recognized (1). It has been assumed that the presence of interstitial oedema associated with the pulmonary congestion sets up a series of events that results in the clinical manifestations of respiratory wheezing, cough, dyspnoea, and, ultimately, the expectoration of blood-tinged fluid when alveolar oedema develops. The precise mechanism (or more likely, mechanisms) responsible for the respiratory distress associated with left ventricular failure has not been fully investigated although a number of factors have been considered. For instance, it has been suggested that oedema of the bronchial mucosa increases resistance to air flow (1), producing a respiratory distress similar to that found in bronchial asthma, hence the term "cardiac asthma". While this proposition appears to be a reasonable one, the precise way in which the bronchial oedema brings about the change in air flow remains unclear.

It is likely that more than one of the following mechanisms are operative.

1. The accumulation of oedema fluid in the interstitial space causes mucosal swelling which impinges on the airway lumen (2).
2. Extrinsic compression on the airway as a result of the development of peribronchial oedema and vascular engorgement (3).
3. Decrease in radial traction as a consequence of the increased "stiffening of the lungs" from the fluid and blood accummulating in the interstitial spaces. As a result the functional residual

capacity of the lungs is reduced (4).

4. Reflex bronchoconstriction (5,6).

The first three of these mechanisms involve changes in local mechanics of the lungs and airways due to accumulation of interstitial fluid. The mechanism last postulated, the presence of a neurally mediated reflex bronchoconstriction during pulmonary congestion, requires further investigation. The present study was designed to examine the presence of such a reflex.

In the series of experiments in this study, pulmonary congestion was produced by elevation of the left atrial pressure brought about by partial obstruction of the mitral valve. Then, the effects of sustained pulmonary venous congestion on the known afferent vagal endings of the lungs and on airway smooth muscle tone were studied. Attempts were made to delineate the afferent and efferent pathways involved in changing the tracheal smooth muscle tone during sustained pulmonary venous congestion.

CHAPTER 2

OUTLINE OF THE REVIEW OF THE LITERATURE

In this section, it is planned to review the literature dealing with the various components which could be involved in reflex bronchoconstriction. In the study of reflex bronchoconstriction, it would not be unreasonable to assume that the afferent nerve endings in the lungs and airways are the receptors that may be involved. However, as pulmonary congestion usually involves elevation of the pressure in the left atrium, the atrial receptors too, may be involved. There is little doubt that the smooth muscle of the airways is the effector organ of the reflex arc. It is likely that the main afferent nerves from the lungs and airways discharging into the vagus nerves form the afferent link while the motor nerve supply to the smooth muscle constitutes the efferent link.

The plan of the literature review in this section is as follows:

1. Since the development of cardiac asthma involves the accumulation of fluid in the interstitial spaces of the lungs and airways, the physiology and pathophysiology of pulmonary fluid exchange, particularly during the development of pulmonary congestion and oedema will be reviewed first.
2. The physiology of the receptors thought to be involved in the refex arc will be reviewed. Two major groups of receptors, i.e., pulmonary and cardiac receptors, will be considered.
3. Finally, the anatomy and physiology of airway smooth muscle and the reflex regulation of the airway smooth muscle will be reviewed.

CHAPTER 3

REVIEW OF PULMONARY FLUID EXCHANGE DURING PULMONARY CONGESTION

Over the last 30 years, starting with the pioneering work of Guyton and co-workers (7), studies on the physiology of fluid exchange in the lungs and of pulmonary oedema has made tremendous advances (8). As the mechanism involved in pulmonary fluid exchange is not the object of this study, it is proposed to review only the following:

A. Factors affecting transvascular (transcapillary) fluid exchange,

B. Factors affecting accumulatation of interstitial fluid in the lungs. Included in this are the pulmonary and bronchial circulations and pulmonary lymphatic flow,

C. Methods of quantifying lung water,

D. Formation of pulmonary interstitial and alveolar oedema,

E. Mechanisms (including experimental techniques) of producing pulmonary congestion and oedema.

A. Factors Affecting Transvascular Fluid Exchange - General Considerations

During the last decade of the nineteenth century, Starling published a number of papers dealing with transvascular fluid exchange and pointed out the importance of intravascular hydrostatic pressure and of proteins in exerting osmotic effects in transvascular fluid exchange (9,10). Probably because of the importance of this work, the mathematical equation describing the way water moves in and out of the lung and other tissues in response to hydrostatic and osmotic forces in microvascular vessels, interstitium and lymphatics is now called the Starling Equation. The final form of this equation,

as shown below, was defined during the first half of this century (11,12,13).

$$Q_f = K_f [(P_{mv} - P_{pmv}) - \sigma_f(\Pi_{mv} - \Pi_{pmv})]$$

where,

Q_f = net flow (rate of transduction) of fluid from blood vessels to interstitial space,

K_f = apparent fluid filtration coefficient,

σ_f = reflection coefficient of proteins,

P_{mv} = hydrostatic pressure in microvasculature,

P_{pmv} = hydrostatic pressure in perimicrovascular interstitium,

Π_{mv} = colloid osmotic pressure in microvasculature,

Π_{pmv} = colloid osmotic pressure in perimicrovascular interstitium.

Though the Starling Equation gives an apparently simple means of determining the fluxes of water between the microvasculature and the surrounding interstitium, there are a number of difficulties in measuring some of the factors involved in the equation, particularly under different physiological conditions.

Classically, it has been thought that filtration occurs at the arterial end and absorption at the venous end of the capillary because of the hydrostatic pressure gradient along the capillary.

In general, microvascular hydrostatic pressure can be estimated by measuring the difference between the pre-capillary and post-capillary pressures, while the plasma oncotic pressure approximates

closely to the microvascular colloid oncotic pressure. Perivascular interstitial hydrostatic and colloid osmotic pressures can be estimated by measurements of hydrostatic pressures in implanted capsules and determining the colloid osmotic pressure of the lymph in these capsules respectively.

K_f, the microvascular membrane filtration coefficient is defined as the conductance to filtered liquid, i.e., the product of the membrane hydraulic conductivity per unit area (Lp), and the exchanging surface area (A). Therefore, K_f is directly proportional to the total surface area of the exchanging vessels and inversely proportional to their thickness.

In experimental studies, it has often been assumed that the reflection coefficient, σ_f, is equal to one, i.e., colloids are not allowed to pass through the capillary membrane. However, it is known that macromolecules do pass the capillary membrane by a number of possible ways: (i) through clefts in the membrane, (ii) pinocytotic processes in the capillary endothelium, (iii) in the presence of a raised hydrostatic pressure, and (iv) damage to the endothelial barrier by toxins. Comparatively smaller molecules appear to pass more freely. Thus, it has been found that the mean lymph to plasma protein ratio may be as high as 0.75 with the larger molecules, e.g. globulin, showing a higher reflection coefficient and a lower lymph to plasma ratio than smaller molecules, e.g. albumin (14). Blake and Staub have suggested a σ_f of 0.8 for plasma proteins (15).

Besides the various factors associated with the Starling Equation, the degree of lymphatic drainage also determines the

accumulation of interstitial fluid. The lymphatics play a key role in removing fluid from the interstitial space, i.e.,

Rate of accumulation = $Q_f - Q_{lymph}$

where Q_f = flow of fluid from blood vessels to the interstitial space and Q_{lymph} = flow of fluid in the lymphatics.

B. Factors Affecting the Accumulation of Interstitial Fluid in the Lungs

In the lungs, microvascular hydrostatic pressure can be estimated with reasonable accuracy by obtaining the mean pulmonary artery pressure (PAP) and the pulmonary capillary wedge or left atrial pressure (LAP). Then microvascular hydrostatic pressure is calaculated from, P_{mv} = LAP + 0.4(PAP - LAP). With regard to the measurement of the perivascular hydrostatic and colloid osmotic pressures there appears to be some unresolved issues. These are discussed further below.

Experimentally, it has been assumed that,

(1) pleural pressures or hydrostatic pressure in implanted capsules are close approximations of the perimicrovascular interstitial hydrostatic pressure (16).

(2) colloid osmotic pressure of lymph collected from the area or the colloid osmotic pressure in the free space of implanted capsules in the area is representative of the interstitial colloid osmotic pressure (17).

However, Staub and other workers have pointed out some inaccuracies in such assumptions (8). Firstly, it was found that, by inserting micropipettes into the perivascular interstitium of the

hilar vessels of dog lungs, the directly measured perivascular interstitial hydrostatic pressure was much more negative than the pleural pressure, particularly at higher lung volumes. Secondly, Staub pointed out that the colloid osmotic pressure of lymph collected from the lungs might be contaminated by lymph from other parts of the body.

K_f, the microvascular membrane filtration coefficient in the lungs, which is directly proportional to the total surface area of the exchanging vessels and inversely proportional to their thickness, has never been measured accurately. It has been suggested that in the lungs, fluid exchange takes place in pulmonary vessels with a thickness of 50 um or less, with the capillary bed accounting for 95% of the total fluid exchange and the remaining 5% accounted for by extracapillary vessels formed by venules and arterioles adjacent to these capillaries (13).

In the lungs, the reflection coefficient, σ_f, does not differ from that of the other organ systems (see pg.6 for further discussion on σ_f).

Pulmonary oedema occurs when Q_f is markedly increased and the pumping capacity of the lymphatic channels is exceeded. In the dog, at rest, lymphatic flow has been measured to be 3-4 ml per hour (18). By extrapolating from animal data, it has been estimated that in the average 70-kg person, the average Q_{lymph} at rest is approximately 20 ml per hour (19). Experimentally, lymph flow rates of up to 10 times control values have been reported (8). The role of lymph flow and formation of pulmonary oedema will be dealt with in a later section (pg.16-17).

Bronchial Circulation

The bronchial arteries and their branches supply blood from the systemic circulation to the conducting airways and extend the entire length of the airways to the respiratory bronchioles (20). In the region of the respiratory bronchioles, overlap exists with blood from the pulmonary artery. Thus, despite the relatively small size of this circulation when compared to that of the pulmonary circulation, changes in the bronchial circulation can be expected to have an influence on the activity of airway receptors.

Of considerable importance in this regard appears to be the distribution of the venous portion of the circulation. Along the length of the airways, the bronchial veins form a communicating network that consists of a plexus on either side of the bronchial muscle linked by transmuscular venous channels. The submucosal venules form a venular plexus which parallels another venular plexus that is formed by larger vessels and is located in the peribronchial connective tissue, outside the cartilaginous plates (20). It is believed that while some of the bronchial venous blood drains into the systemic venous system, a significant proportion drains into the pulmonary veins (20).

This arrangement of the venous system could have a significant role in the production of bronchial mucosal oedema. Elevation of pulmonary venous pressure can result in increases in pressure of the bronchial venous system. Also, contraction of bronchial smooth muscle may interrupt many, if not all, communicating channels connecting the venous plexuses on either side of the muscle layer, and interfere with the venous drainage from the bronchial capillaries and

submucosal veins (20). Whatever the initiating cause, engorgement of the submucosal veins could lead to submucosal oedema and possibly affect the activity of the afferent nerve endings located in this area.

Recent studies have shown the importance of the bronchial circulation as a possible source of interstitial oedema. This effect is related to the concept of "leaky bronchial vessels". It has been shown that administration of histamine, by whatever route, transiently caused transduction of fluid across the bronchial venules while leaving the pulmonary microcirculation unaffected (21). This mechanism has been postulated to be due to stimulation by histamine of contractile fibrils in the endothelial cells lining the bronchial venules and capillaries, causing the interendothelial junctions to be stretched, and resulting in gaps which allow fluid to enter the interstitial space (22).

This finding suggests that the venular smooth muscle was not responsible for the leakage. It can be hypothesized that, peribronchiolar oedema as part of the exudative process due to local injury such as bronchitis, could occur as a result of the leaky bronchial venules present in the submucosal bronchial venular plexus.

C. Methods of Quantifying Lung Water

Lung water can be quantified by either direct or indirect methods.

Direct Methods

All these methods involve examination of the lungs directly. Examples of these methods are:

(1) Repeated weighing of an isolated perfused lung as an indication

of changes in lung water based on changes in weight (23). The main drawback is that in the isolated lung, there is interruption of the lymphatics and therefore, it does not reliably reflect a physiological change.

(2) Measuring the ratio of wet-to-dry lung weight is the standard method in the quantitative assessment of lung water. In this, all blood is drained from the vessels and the lung is weighed, then dried and weighed again (7). One of the disadvantages of this method is that there is usually an amount of retained blood in the smaller blood vessels despite efforts to drain it. Also, it can be used only to determine the end-result of a prolonged experiment and not for repeated observations at short intervals.

Indirect Methods

A number of indirect methods are used, some of which are listed below:

(1) Vascular injection of freely diffusible tracers (Chinard technique).

This method involves derivation of simultaneous indicator-dilution curves from measurements of a detectable tracer substance that remains in the vasculature and one that diffuses quickly into the interstitial space (24). Usually radio-labelled red blood cells or plasma albumin is used for the former and a water tracer (^3H_2O) for the latter. This method has a sound theoretical basis, is safe and is easily repeatable. However, Staub (8) has shown that, using this method, the extra-vascular lung water volume in normal resting supine man averages about 2.8 ml/kg body weight, whereas the corresponding value in "normal"

lungs examined post mortem was 5.8 ml/kg body weight. Thus, it appeared that Chinard's technique detected only half the lung water present. A number of reasons have been suggested to explain this discrepancy: (i) the distribution of blood flow may be uneven so that the tracers do not reach all parts of the lungs (25) and (ii) the water tracer such as tritiated water (^3H_2O) may not equilibrate with tissue water in one single transit interval and problems with recirculation arises (13). If "heat" is used as a tracer, as in the thermal dilution method, overestimation of lung water may occur because heat may reach non-aqueous elements of the lung (13). Attempts are being made to modify the method and to correct for inaccuracies in the indicator dilution curves, e.g. by the use of correction factors.

(2) Inhalation of soluble inert gases (Cander and Forster technique).

This is based on the same principle as the indicator dilution technique. A soluble inert gas such as acetylene or nitrous oxide is inhaled and the amount of lung water is estimated based on the inspired volume, time taken, concentration and solubility coefficient of the gas (26). This method suffers from inaccuracies due to lung inhomogeneities and maldistribution of ventilation in the lungs and tends to underestimate lung water.

(3) Substraction of gas volume from lung volume.

In this method, thoracic volume is calculated from postero-anterior and lateral radiographs. Gas volume is measured by a body plethysmograph. The difference reflects the tissue volume of the lungs (minus the volume of the heart, mediastinum and subphrenic structures) (27). The method suffers from the

disadvantage that the radiographic and body plethysmographic measurements are not made simultaneously. Moreover, the measurements are made with the subject in different postures.

(4) Chest radiography

This method is used routinely in clinical practice in estimating pulmonary oedema. It has been found by monitoring lung water by the double indicator method that chest radiography becomes abnormal when lung water increased by 30-80% (28). One study, examined the presence of basal horizontal lines on chest radiographs in patients with elevated left atrial pressure, and showed that these lines were invariably present when the left atrial pressure was 24 mm Hg or higher (29). However, the main drawback of the technique is that it is not quantifiable.

(5) Densitometry

This method involves assessing the changes in radiographic density using a densitometer to indicater changes in lung water. It is no more sensitive than a critical visual examination of a chest radiograph (30).

(6) Computerized tomography

This method gives an absolute measurement of density (31). However, it has been found that, in oedema, as the extravascular water volume rises, the computed pulmonary blood volume and air volume may fall, thus complicating interpretation of the results. It has been suggested that it may be more advantageous to select peripheral areas of lung, away from major blood vessels to avoid the above complication.

(7) Impedance plethysmography

Changes in transthoracic impedance have been used previously to detect changes in lung water and pulmonary oedema. However, its sensitivity suffers because of the wide range of impedance with changes in posture and its inability to distinguish vascular from extravascular fluid (32).

(8) Other methods

A number of techniques measuring the volumes of plasma, extracellular water and total lung water in the steady state are being developed using radio-labelled substances, e.g., albumin, and externally detecting these substances by scintigrams (33). Another method involves using positron emission tomography to measure lung water (34). These methods are still in the state of development and their value remains to be established.

The methods of estimating lung water decribed above range from those used only in the animal laboratory to those used in the usual clinical setting. It is obvious therefore that, in any given situation, a choice has to be made as to which technique would be the most appropriate depending on the circumstances. To date, not one method has emerged as the "gold standard" with which the others could be compared and standardized.

D. Formation of Pulmonary Interstitial and Alveolar Oedema

From the discussion on the bronchial circulation (pg.9-10), it would appear that bronchial oedema and pulmonary oedema may be two separate entities, one being associated with the proximal airway and the other with the distal. This proposition could be supported to

some degree by the clinical observations that cough and wheeze would be related to bronchial mucosal oedema and the expectoration of blood -tinged fluid more related to the advanced stages of pulmonary oedema. However, excepting the extra-pulmonary airways, the "interstitial space" of the lungs is in intimate contact with the intra-pulmonary airways as well as the alveoli. Changes in the pulmonary circulation leading to changes in the interstitial fluid content are likely to have profound influences on the bronchial tree, firstly via local mechanical effects referred to previously on page 1, and also because of the close connection between the pulmonary and bronchial veins (pg.9-10).

Functionally, for efficient gas exchange, the alveoli and the interstitium between the alveolar epithelium and the capillary endothelium need to be as clear of fluid as possible. Thus, if excessive fluid accumulates in the interstitial space or if fluid accumulates in the alveoli, gas exchange will be impaired and a pathological process exists.

Anatomical Considerations

Anatomically and functionally, the air in the alveoli is separated from the blood in the capillaries by 3 distinct layers, the alveolar epithelium, the interstitium and the capillary endothelium. The capillary endothelial cells join to each other by abutment, interdigitation or overlap of their cytoplasmic projections to form a continuous cytoplasmic tube. At the junction of the cytoplasmic projections are clefts which communicate between the capillary and interstitium. These clefts are of varying sizes, averaging 40 μm in width, and because they widen with relatively small increases in

vascular pressure, they are referred to as "loose junctions".

On the other hand, the epithelial lining of the alveoli is predominantly made up of large squamous cells, type I pneumocytes, with thin cytoplasmic projections, and a smaller number of granular type II pneumocytes. In contrast to the "loose junctions" of the capillary endothelium, the projections of the epithelial cells overlap, abut and fuse to obliterate the "junctional clefts". These intercellular fusions require much greater distending forces than do the capillary endothelial junctions and are referred to as "tight junctions", and their function appears to be to prevent easy flooding of the alveoli.

The interstitium has been divided into 3 compartments: (i) the alveolar interstitium in the alveolar septa, (ii) the extra-alveolar interstitium surrounding the large airways and blood vessels and (iii) the junctional interstitium, lying between the other two compartments. The alveolar interstitium is formed partially of opposed epithelial and endothelial basement membranes and partially of looser tissue containing bundles of elastic and collagen fibrils and fibroblasts. This part of the interstitial space has been referred to also as the "tight interstitial space". The extra-alveolar interstitium consists of a loose network of connective tissue surrounding the pulmonary vessels and bronchi. The junctional interstitium is described as the point where three alveolar septa meet and this forms a connection with the alveolar interstitium (13).

Stages of Development of Pulmonary Oedema

The following is a description of the development of pulmonary oedema, which develops initially in the interstitial parenchyma.

However, as more fluid accumulates in the interstitial space, it will have an influence on the lumen of the bronchial tree (pg.13). The stages of the development of pulmonary oedema described below is an attempt at explaining the progression of this pathological process.

The development of pulmonary congestion and oedema has been divided into three stages (35). During the initial phase or Stage 1, there is an increase in transfer of liquid and colloid from the capillaries through the interstitium. There is no increase in interstitial volume because of an equal increase in lymphatic flow due to increased lymphatic pumping.

Stage 2 is approached when the pumping capacity of the lymphatics is reached or exceeded. There is an accumulation of fluid and colloid in the more compliant junctional and extra-alveolar interstitial spaces.

When the filtered load is further increased, the capacity of the loose interstitial space is exceeded. There is distension of the tight interstitial spaces and the pressure may be sufficient to disrupt the tight junction of the alveolar membrane, leading to alveolar oedema. The fluid initially accumulate at the corners of the alveolar-capillary membranes (Stage 3a) followed by alveolar flooding (Stage 3b) (35).

E. Mechanisms Producing Pulmonary Congestion and Oedema

The factors leading to the development of pulmonary congestion and oedema can be generally grouped into (a) imbalance of the Starling forces, (b) altered alveolar-capillary membrane permeability and (c) lymphatic insufficiency. The role of the bronchial

circulation in producing bronchial mucosal oedema has already been reviewed (pg.9-10).

(a) Imbalance of the Starling Forces

These may present as:

Raised Left Atrial Pressure (increased P_{mv})

Clinically, mitral stenosis and left ventricular failure are the common disease conditions associated with a raised left atrial pressure. Experimentally, raised left atrial pressure can be produced by partial obstruction of the mitral valve (6) or partial occlusion of the aorta (7). The length of time required for these manoeuvres to initiate pulmonary oedema is not known. Clearly, this is related to the extent as well as the duration of raised left atrial pressure. Guyton and Lindsey showed that left atrial pressure of less than 25 mm Hg did not produce pulmonary oedema (7). Rossall & Gunning showed that the presence of basal horizontal lines on chest radiographs invariably appeared in patients with left atrial pressures greater than 24 mm Hg. These lines were attributed to an "increase in bulk of the interalveolar septa" due to increased tissue fluid (29).

Widdicombe (36) avoided the development of pulmonary oedema by raising left atrial pressure by 30 mm Hg for only 2-3 minutes. Guyton and Lindsay observed that at normal plasma protein concentrations, pulmonary oedema occurred after 30 minutes, only when the left atrial pressure exceeded 25 mm Hg (7). Interestingly, Erdman et al suggested a hyperbolic relationship between vascular pressure and increases in lung water (12).

Increased Pulmonary Arterial Pressure (increased P_{mv})

Clinical examples of sudden increases in pulmonary arterial

pressure associated with pulmonary oedema are situations where there is an acute volume overload such as overinfusion of intravenous solutions. In the laboratory, increased pulmonary arterial pressure can be produced by aorto-pulmonary shunts and infusion of large amounts of crystalloid solutions (37).

Decreased Plasma Colloid Osmotic Pressure (decreased Π_{mv})

In the clinical situation, decreased plasma colloid osmotic pressure more often produces peripheral oedema than pulmonary oedema because of the higher arterial pressure encountered in the systemic circulation. Experimentally, acute pulmonary oedema has been produced by replacing plasma-containing proteins with crystalloid solutions (37). The mechanism of production of this form ofpulmonary oedema is probably multifactorial.

(b) Altered Alveolar-Capillary Permeability

Altered alveolar-capillary permeability has been the postulated mechanism in producing pulmonary oedema in a large number of clinical conditions sometimes grouped under the condition "adult respiratory distress syndrome" (ARDS). Agents that bring about this change are usually toxins from bacterial, viral or parasitic sources, chemicals such as phosgene and chlorine, and autocoids such as histamine and bradykinin.

(c) Leaky Bronchial Vessels

The concept of "leaky" bronchial vessels has been reviewed to earlier in the section on the bronchial circulation (see pg.9-10). Using histamine, bradykinin and serotonin, Pietra and Fishman (20) have come to the following conclusions regarding "leaky bronchial vessels":

1. Certain agents, traditionally believed to cause pulmonary leakage, elicit bronchial venular leakage instead.
2. This effect was independent of the route of administration.
3. In the lungs, the affected vessel are systemic venules, i.e. the bronchial circulation.
4. That contraction of the bronchial venular smooth muscle was not responsible for the leakage (20).

(d) Lymphatic Insufficiency

This may be brought about by a surgical intervention, e.g., post lung transplant or disease conditions such as lymphangitic carcinomatosis. Experimentally, surgical interruption of the lymphatic channels has not been used to produce pulmonary oedema. However, elevation of the systemic central venous pressure by 15 to 25 mm Hg has been shown to cause lymphatic obstruction by applying an afterload to the major lymph vessels, and increased lung water (38).

Uhley et al (18) described a method of collecting lymph from some areas of the lungs by preparing an isolated venous pouch from that part of the right external jugular vein into which the pulmonary lymphatic vessels drain in dogs.

It has been pointed out previously (pg.16-17), that during the earlier stages of pulmonary oedema, the tendency of the interstitial fluid to accumulate is prevented by increased lymphatic drainage. Guyton and Lindsey (7) demonstrated that below a critical pressure of 25 mm Hg in the left atrium of the dog, lymphatic drainage was sufficient to prevent fluid accumulation. It is conceivable that where the lymphatic drainage from the lungs is obstructed, left atrial pressures below this critical value could likely produce a

form of oedema in the lung.

(d) Other Mechanisms

A number of disease conditions have been found to lead to pulmonary oedema, e.g., that associated with high altitude, narcotic overdose and eclampsia. The mechanisms in these situations have not been completely clarified, probably because of the lack of suitable animal models.

Summary on Pulmonary Fluid Exchange

(1) Since the pioneering work of Starling, the study of transvascular fluid exchange has made a tremendous progress, particularly in the last three decades.

(2) The Starling Equation is widely used to describe the transvascular movement of lung water.

(3) In order to study the physiology and pathophysiology of pulmonary congestion and oedema, as well as for clinical purposes, a large number of methods have been developed to measure lung water. However, none has been found to be applicable in the majority of situations.

(4) The causes of pulmonary congestion and oedema have been extensively investigated because of their importance in the management of clinical pulmonary oedema.

CHAPTER 4

REVIEW OF THE PHYSIOLOGY OF THE PULMONARY SENSORY RECEPTORS

The literature of the pulmonary sensory receptors discharging into the vagus nerves is extensive. A number of papers and reviews have discussed at length the possible roles of these receptors in the regulation of respiration and the various cardiorespiratory reflexes associated with these receptors (39,40,41). There is little information concerning the role of these receptors in cardiac asthma. Since the aim of the present study is to investigate a possible sensory mechanism involved in cardiac asthma, it is intended to discuss the general physiology of the pulmonary receptors with an emphasis on the activity of these receptors during pulmonary congestion and oedema where appropriate. It is planned to present reviews of:

A. Early studies dealing with pulmonary reflexes associated with the vagus nerves and how these studies gave rise to the concept of pulmonary receptors before they were identified.

B. Early electrophysiological studies on the pulmonary receptors and their differentiation into subtypes.

C. Detailed reviews of the of the properties of the different pulmonary receptors.

A. Review of the Historical Studies relating to Pulmonary Reflexes associated with the Vagus Nerves.

During the nineteenth century, the origin of the rhythmicity of respiration and the role of the vagus nerves were actively pursued and the concept of a "respiratory centre" was advanced (42). Work

by Krimer, Traube, Rosenthal and Hall, among others, gave rise to the concept of a constant drive from a respiratory centre. The effect of the vagus was thought to be excitatory to breathing via a reflex action mediated by the level of alveolar carbon dioxide (42).

In 1868, Hering and Breuer described a reflex, now bearing their names, in which inflation of the lungs stopped respiration in inspiration. They inferred that inflation of the lungs mechanically stimulated nerve endings in the lungs and the resulting impulses which ascended in the vagi were inhibitory to inspiration (43). Thus, the concept of a negative feedback was incorporated into the theory of control of respiration.

In 1889, Head's paradoxical reflex was described. It was found that when the ice-cooled vagus nerves of the rabbit were being rewarmed, moderate inflation of the lungs evoked a vigorous diaphragmatic contraction (44,45). This "paradoxical reflex" was regarded as the reverse of the Hering-Breuer reflex. However, very often Head's paradoxical reflex has been equated with the "gasp reflex" (39), defined as a brief powerful contraction of inspiratory muscles evoked by a large, abrupt inflation (46). It has been demonstrated also that the paradoxical reflex could be evoked when the vagi were cooled to between 3 and 5^{o}C (47,48), a finding which would be of considerable interest when the afferent limb of this reflex is considered. This is because at 3 to 5^{o}C, non-myelinated rather than myelinated vagal afferent fibres would be involved due to the preferential blocking in conduction in the myelinated fibres at this temperature.

Further work was done on the respiratory system by Hammouda and

Wilson in the early part of this century. In a series of experiments, these investigators showed that tracheal distension led to a reflex slowing of respiration. It was shown that the vagus had a dual inhibitory and excitatory influence on respiration, depending on the state of inflation or deflation of the lungs and that the effect could be blocked differentially. It was concluded that the excitatory and inhibitory effects were due to the stimulation of different types of nerve fibres in the vagi (49,50,51).

Brodie and Russell studied the effects of chemical stimuli (blood serum) on the respiratory rhythm. They observed that reflex respiratory arrest occurred as a result of this stimulus and concluded that this effect was due to stimulation of the receptors in the lungs (52). The authors had in fact described what is now called the "pulmonary chemoreflex" which usually includes the triad: apnoea (sometimes followed by rapid shallow breathing), bradycardia and hypotension (53).

Thus, by the early part of this century, the stage was reached where investigators were able to proceed to examine the nerve fibres in the vagi into which the receptors in the tracheobronchial tree and the lungs discharge and to draw some functional and anatomic conclusions about these receptors.

B. Review of the Early Electrophysiological Studies of Pulmonary Receptors which Discharge into the Vagus

In 1933, Adrian recorded impulses from single afferent nerve fibres in the vagus and studied the effects of these afferent impulses on respiration. He related these afferent impulses to receptor endings in the lungs and described their stimulation during

lung inflation (54). He likened these receptors to stretch receptors in skeletal muscles, both types of receptors being slowly adapting and having an inhibitory action on limiting the activity which brought about their stimulation. Though Adrian did not mention the Hering Breuer reflex by name, the inhibitory reflex action of these lung receptors appeared to be responsible for this reflex. This paper was the first description of "slowly adapting pulmonary stretch receptors". The study also described other afferent impulses, such as those which showed a cardiac rhythm and others which showed rapid adaptation when stimulated by lung collapse brought about by suction of the airways. Not much was mentioned of the receptors which adapted rapidly in this paper (54).

In the cat, rapidly adapting receptors were first described in detail by Knowlton and Larrabee in 1946 (55). These authors studied the rate of adaptation of pulmonary stretch receptors (referred by the authors as volume receptors) over a fixed interval of time during maintained lung inflation and the idea of an "adaptation index" given by the formula below was described:

$$\text{Adaptation index} = \frac{\text{Peak frequency} - \text{Average frequency during 2nd second of inflation}}{\text{Peak frequency}} \times 100\ \%$$

Receptors with adaptation indices less than 55 % were classified as slowly adapting receptors and these corresponded to the slowly adapting receptors described by Adrian (54). Those with adaptation indices greater than 80 % were called "rapidly adapting receptors". A small number of receptors (8%) had adaptation indices between 55 and

80% and these were called "intermediate adapting receptors". The authors added that these could be assigned to one or the other of the two groups by their individual discharge characteristics. No explanation was given as to why the receptors behaved in this way other than the mention that no intermediate adapting receptors were recorded in animals with closed chests, implying that this might have been an artifactual finding. A feature not defined in this paper was that the authors did not describe precisely how the "peak frequency" was measured and from when the second second of inflation was measured.

To date, because of this omission, there has been no universally accepted definition of the adaptation index and no standard method of causing lung inflation during measurement of the index. However, the authors suggested that the role of the rapidly adapting receptors was to reinforce the depth of deep inspiration which has been initiated through other mechanisms. These were interpreted by Widdicombe (46, 56) as Hering Breuer deflation reflex and Head's paradoxical reflex.

Widdicombe repeated the work of Knowlton and Larrabee and attempted to clarify the definition of "adaptation index" (57). The author decided to use the point of peak activity as the start of the 2nd second of inflation. This point, which coincided usually with the peak inflation, was not necessarily the 2nd second from the beginning of inflation. It was definitely not the 2nd second following completion of inflation. The author also classified the receptors as rapidly adapting (adaptation index >70 %) and slowly adapting (adaptation index <55 %). A group of "intermediate receptors" was also described and the majority of these were found in the

mediastinum while others of this group were uniformly distributed in the tracheobronchial tree (57). The issues raised in the preceding paragraphs concerning the different rates of adaptation will be discussed in the section on rapidly adapting receptors (pg.37-40).

In 1955, Paintal reported observing low amplitude action potentials from multifibres of the vagus nerve, which subsequently were found to originate from the lung, following injection of phenyl-diguanide into the right atrium of the cat (58). These afferent nerve fibres were found to be non-myelinated and the receptors which discharge into them could be stimulated by capsaicin and phenyl-diguanide. One type was postulated to lie in the lung parenchyma adjacent to the pulmonary capillaries and alveoli. Hence, they were called "Juxta-pulmonary capillary" or "J" receptors. They were thought to be responsible for the reflex effects following pulmonary congestion or oedema (59). The other type was sensory receptors discharging into non-myelinated vagal afferent fibres located elsewhere in the tracheobronchial tree. These have been called "bronchial C-fibre receptors" (60).

Thus, four types of sensory receptor endings have been identified electrophysiologically in the lungs and tracheobronchial tree. These will be reviewed in greater detail below.

C. Review of the Individual Types of Pulmonary Receptors.

Slowly Adapting Pulmonary Receptors (SAR)

These are mechanoreceptors which were originally studied by Adrian (54). Of the 4 types of pulmonary receptors, the SAR have

patterns of discharge which most closely relate to expansion of the lungs (40,41). Their discharges during inspiration provide the afferent input that form the off-switch in the Hering-Breuer inflation reflex (61). It has also been shown that SAR cause relaxation of the tracheobronchial smooth muscle and dilatation of the airways (62). Studies of conduction velocities and vagal blockade by cooling indicate that these receptors discharge into myelinated afferent fibres (40,63,64).

Distribution of SAR

A number of studies have reported the distribution of SAR in different animal species (54,55,57,65). The receptors are usually located by introduction of a probe into the airway lumen and stimulation of the mucosa while single fibre action potentials are being recorded. In open chest animals, an alternate method, is to probe the airways externally to elicite a response. It has been suggested that SAR are stimulated when the structures containing them are stretched (66). At first sight there appears to be no close agreement among the investigations regarding the distribution and location of the SAR. One report (67), suggested that most of the SAR in the rabbit were in or near the visceral pleura on the basis of directly anaesthetising this structure. However, this was challenged by Widdicombe (68) who found that removal of the visceral pleura did not affect the activity of these receptors which could still be activated by direct probing of the airway mucosa.

There is now general agreement that the SAR are mainly found in the airways, particularly at the tracheal bifurcation and extra-pulmonary bronchi (69,70). Systematic investigations have shown that

39% of SAR in the cats (71), 56% in dogs (70) and 37% in rabbits (72) are located in the extrapulmonary airways. However, another study found that only 17% of SAR were in extrapulmonary airways (73). There is some controversy as to whether SAR are located in the respiratory bronchioles and alveoli. One study found that the smallest airway shown to have SAR had a diameter of 0.3 mm in the collapsed lung, which corresponded to the terminal bronchioles (74). However, another report suggested that SAR were found in sites peripheral to the respiratory bronchioles, i.e., in the alveoli of guinea pigs (75). At present, there is no conclusive evidence to exclude the possibility that some SAR may be located in the respiratory bronchioles and alveolar ducts. The problem may be due to the close proximity of the respiratory bronchioles and alveolar ducts in the lung parenchyma. In practice, it would not be possible for the exploring probe to localise precisely the SAR to one or other of the structures.

Morphology and Location of SAR within Airway Walls

The morphology of afferent nerve endings in the tracheobronchial tree and lungs has been studied by light and electron microscopy (69,76). Afferent nerve endings are identified by their morphological similarity to afferent nerve endings elsewhere, e.g. in skin (77). The SAR consist of myelinated fibres whose end formations are closely associated with airway smooth muscle. Just before losing its myelin sheath, the axon enlarges and splits into terminal branches which end as lanceolate terminals. Many mitochondria, found in the axon at its termination and in the terminals of the branches, characterize these as afferent endings. The branch endings are bound to connective tissue elements between the lamina propria and smooth muscle layer,

and are attached to the underlying smooth muscle by bands of elastin and collagen. They are orientated in the long axis of the airways (76). Degeneration experiments confirm that these fibres are vagal afferents (78).

One study proposed that SAR endings may be located in superficial layers of the bronchial mucosa on the basis that radio-labelled bupivacaine, which blocked the inflation reflex in rabbits studied, was later found mostly in layers superficial to the basement membrane (79). However, a small amount of bupivacaine was also found within the smooth muscle layer. Thus, this study did not demonstrate conclusively that SAR were located also in the superficial layers of the airway mucosa.

Patterns of Discharge of SAR

The pattern of discharge of SAR has a temporal relationship to respiratory movements (61,80). The rate of discharge has a regular respiratory modulation that increases during spontaneous inspiration from either a low value of activity, or no activity, at end expiration. These discharges have been described as from low threshold or high threshold SAR respectively (41) or as "tonic" and "phasic" discharges (81). It has been proposed that these different patterns reflect differences in the mechanical environment of the receptors (73,82). In other words, low and high thresholds imply an intrinsic difference in the receptors' level of activation whereas their different behaviour reflects a different degree of stimulation related to mechanical factors (41,75,82,83). Between 27 to 60% of SAR in different species studied (41,75,82,83) are active at end-expiratory volume or functional residual volume and it has been

found that these SAR are generally located in the larger, extra-pulmonary airways. Higher threshold SAR, on the other hand, usually do not reach maximum frequency, until distending pressures reach 30 cm H_2O, near total lung capacity, and they are mainly intrapulmonary (82). The differences between the two types of SAR may be due to mechanical factors. In larger extra-pulmonary airways, the same transmural pressure (as applied to the smaller intra- pulmonary airways) will result in a greater stimulus to the SAR because of a larger circumferential tension as predicted by Laplace's law (78)

Occasionally, a SAR may be found to have its maximum discharge during expiration. Such receptors are located usually in the extra-thoracic trachea where transmural pressure is positive during expiration (78). Other SAR may be found to have a cardiac modulation due to their proximity to the heart or other pulsating vascular structure (54). It has been suggested that low threshold SAR regulate respiration during eupnoea, higher threshold SAR regulate T_I during hyperpnoea while those with expiratory activity regulate the duration of T_E (84).

Another feature of SAR discharge is the slow adaptation following a maintained lung inflation, as originally described by Adrian (54). The pattern has been described as a characteristic rapid decline immediately following peak lung inflation that slows progressively into a sustained discharge with a regularity of interspike distances not found in other types of receptors (55,57). This response has been attributed to the transduction property of the SAR in responding to transmural pressure changes instead of to volume changes. Immediately following a fixed-volume lung inflation, the lungs distend further

leading to a drop in transmural pressure coinciding with the rapid decline in SAR activity immediately following peak inflation (55,57). This hypothesis is supported by reports that constant pressure inflations are more effective than constant volume inflations in maintaining a given rate of discharge in SAR (55).

The mechanism of stimulation of SAR has been postulated to be due to changes in circumferential tension in all of the airways. The orientation of smooth muscles in the trachea and extrapulmonary bronchi is strictly transverse and a tension applied along this axis causes discharge of the SAR whereas a longitudinally applied force, perpendicular to this axis fails to stimulate the SAR (85). However, in the peripheral airways, where the smooth muscle fibres lie in an oblique orientation, this hypothesis cannot be tested directly, and here, it may well be that both longitudinal and transverse tension in the airway causes activation of the SAR.

Responses of SAR to Other Stimuli

(1) Carbon Dioxide

Increasing the level of carbon dioxide has been documented to inhibit SAR activity, particularly when the initial level of carbon dioxide was low (alveolar pCO_2 < 30 mm Hg) (86,87), but the physiological significance of the finding remains unclear. It was also found that, among the intrapulmonary SAR, only bronchial SAR are susceptible to CO_2 inhalation (88) and that increasing the concentration of CO_2 in the blood failed to have an effect (88). A number of proposals have been suggested to explain the mechanism of action of CO_2 on SAR. It has been proposed that the changes may be mediated by changes in airway smooth muscle tension (87), changes

in hydrogen ion concentration (89), release of protein-bound Ca^{++} (90), or as a general characteristic of neural function particularly in a hypocapnoeic environment (87). However, none of these proposals could explain satisfactorily the mechanism of action of SAR inhibition by CO_2, and the role of SAR in the "pulmonary CO_2 reflex" remains to be evaluated.

(2) Pulmonary Congestion

Pulmonary congestion has been shown to sensitise SAR, a finding interpreted as caused by a reduction in lung compliance, since a higher transpulmonary pressure is required for any given lung volume change, leading to a greater SAR discharge (91).

(3) Pulmonary Oedema

The threshold for SAR activation is lowered by pulmonary oedema and there is a greater rate of discharge at any inflating volume (36,91). It has been suggested that, in the presence of alveolar oedema fluid, lung inflation would cause a disproportionate distension of the airways (36).

(4) Pulmonary Microembolism

SAR have been shown to be activated by microemboli in the pulmonary circulation in some studies (92,93), but not in others (94). It has been suggested that, where there was activation, it was due to a decrease in lung compliance and to pulmonary vascular obstruction (92)

(5) Atelectasis

The initial reinflation following atelectasis of a lung was found to markedly decrease the volume threshold for activation of the SAR (36,95). It was interpreted as meaning that collapsed alveoli require

a critical pressure to be reopened, resulting in a greater distending pressure across the airways during reinflation.

(6) Responses to Chemicals

SAR are stimulated by a few chemicals, none of which stimulate the SAR selectively. Low doses of vertrum alkaloids sensitise SAR and higher doses cause continuous high-frequency firing (68,96).

SAR are stimulated by histamine and acetylcholine indirectly due to contraction of the airway smooth muscle in response to these chemicals (68,81,97). Chemicals like capsaicin and phenyldiguanide do not have any effects on SAR if ventilation is kept constant (98).

High concentrations of volatile anaesthetics like halothane, ether, chloroform and trichloroethylene first increase and then abolish SAR discharge (48,97,99). This inhibition is selective and does not affect non-myelinated afferents (97). In rabbits (100), but apparently not in other species (57), high concentrations of SO_2 abolish SAR activity selectively. This finding could be used to examine selectively the relative contributions of SAR and RAR in some studies.

Rapidly Adapting Receptors (RAR)

This group of pulmonary receptors have been known by various names since its original description by Knowlton and Larrabee (55). This is because of the various reflexes attributed to this group of receptors. They have been called "cough" and "irritant" receptors because of the striking responses of some of these receptors to respiratory irritants such as ammonia, dust and cigarette smoke (101,102,103), substances which evoke cough and other protective

responses from the tracheobronchial tree (57). They have also been referred to as "deflation" receptors because of their stimulation by forced deflation (72,104), although this term is seldom used nowadays as it includes receptors discharging into non-myelinated fibres (58). The generally accepted term for this group of receptors is "rapidly adapting receptors" because of their very rapid adaptation to a maintained lung inflation, producing a brief, irregular burst of activity at peak inflation, the frequency of which decreases rapidly within one second to 20% or less of the initial discharge (see adaptation index, pg.25). Conduction velocity studies and cold vagal blockade indicate that these receptors discharge into myelinated fibres in the vagus nerves (40,63,64).

Distribution of RAR

It is thought that RAR are not as numerous as SAR in the several animal species studied (55,75,93,105). However, in the cat, Widdicombe (57) and Knowlton and Larrabee (55) found that nearly 47% of myelinated vagal fibres which were stimulated by large lung inflations originated from RAR. In rabbits, another study found that the ratio of RAR to SAR was only 1:4 (72).

Morphological and physiological studies indicate that RAR are mainly distributed in larger airways, particularly in the trachea and main bronchi and at branching points (69,106). Unlike SAR, which are distributed in relation to airway smooth muscle only, RAR appeared to be distributed around the circumference of the airway, and the area of innervation of one unit of RAR may extend across two cartilage rings (107). In the dog, it was also found that 33% of spontaneously active RAR were in the extrapulmonary airways, mostly between the

carina and hilum, 67% were in the intra-pulmonary airways, mostly in the large bronchi and only 5% in bronchi with diameters smaller than 1 mm (107). RAR have not been localised beyond bronchioles with diameters of 0.3 mm, suggesting the absence of RAR in the perialveolar region (respiratory bronchioles, alveolar ducts and alveoli) (74).

Morphology and Location of RAR in the Airway Walls

Despite the evidence for the existence of the RAR in the airways, there are few actual histological studies of these receptors (108,109). Epithelial nerve endings, thought to be RAR, have been identified in the airways of several mammals (69,106,110, 111,112). In 1943, Elftman (69), described a receptor in a dog that has since then been widely referred to as an example of a RAR. The epithelial endings described consisted of the myelinated axons of the receptors branching extensively over a wide area of the mucosa, i.e., the terminal arborizations ramify in the tracheobronchial mucosa, frequently at points of bronchial branching. Some of the terminals were seen passing towards the mucosal surface between the columnar cells of the epithelium (69).

These endings have been examined using electron microscopy. The endings, seen between the epithelial cells, both intra and extra-pulmonary, were shown by degeneration studies to be afferent fibres (78). However, no evidence is available at present to positively differentiate these endings from other receptor types such as SAR endings or non-myelinated afferents as these endings are not always myelinated.

Stimulation of some RAR by light touch and inhaled gaseous or

powdery irritants suggests a superficial location for the nerve endings (107), e.g., between epithlial cells as described above. However, it was found that, after removal of the superficial mucosal lining, the underlying layers of the airways still responded to inflation and deflation, suggesting that the receptor terminals are distributed in different layers of the airway wall (106,110,112). This complex branching structure of the receptor has been compared to cutaneous mechanoreceptors. In the latter, the irregular patterns of discharge are believed to indicate spike generation at multiple terminal sites (77). This feature is found also in RAR.

Patterns of Discharge of RAR

The RAR, as originally described by Knowlton and Larrabee, were described as having a higher threshold, a more rapid rate of adaptation and a more irregular pattern of discharge than the SAR (55). With lung inflation, the pattern of RAR activity, though generally related to the respiratory cycle, shows considerable variations in the number and timing of impulses in each cyle. The adaptation index (see pg.25) was introduced to differentiate RAR from SAR. However, as pointed out earlier, there is no universally accepted method of deriving the adaptation index. This is because of different ways of interpreting the original description by Knowlton and Larrabee (55) and basically involves two practical points.

The first is the method of bringing about a "maintained lung inflation". For example, a maintained lung inflation could be brought about by rapidly inflating the lungs with a volume usually equal to 3 times the tidal volume using a calibrated syringe (55,57) or the lungs could be inflated by a step-wise method by occluding the

expiratory line of a "constant volume" ventilator for 3 ventilatory cycles and then switching off the ventilator at peak inspiration (113). A number of studies have avoided the problem by using a constant pressure ventilator (114,115).

The second point is the definition of the 2nd second of inflation. This could be interpreted to mean the 2nd second of inflation as measured from (i) the beginning of inflation, (ii) end of inflation, (iii) the instant of peak frequency and (iv) the 2nd second after the instant of peak frequency. How this 2nd second of inflation is defined could substantially affect the adaptation indices of the receptors under study, particularly those designated as "intermediate receptors" (55,57). For example, Widdicombe (51) defined the 2nd second of inflation as starting from the instant of peak frequency. Most other papers did not define this (105,106, 116,117). The definition of "peak frequency" may also be problematic in practice as it could mean the reciprocal of the shortest interspike interval or the frequency of impulses measured over a finite time interval at the time of highest frequency. Finally, there was no agreed value of the adaptation index which could be regarded as "rapidly adapting". For example, Knowlton and Larrabee (55) originally used 80% and Widdicombe (57) proposed 70% for such a purpose, but, in both papers, the authors also accepted as "rapidly adapting" those intermediate receptors which have the discharge characteristics of RAR.

A well known example of a rapidly adapting receptor in the somatic nervous sytem is the Pacinian corpuscle, which is located in the subcutaneous tissue and is sensitive to vibration. It appears

that in this case, adaptation depends on the non-neural accessory structure which is in the form of concentric layers of connective tissue surrounding the central unmyelinated axon. It is hypothesized that a steady stimulus applied to the outermost layer deforms the inner axon. Then, transverse slippage occurs between the layers of the accessory structure so that, with time, the effective stimulus reaching the axon decreases. Removal of this accessory structure converts the Pacinian corpuscle into a slowly adapting receptor (118).

As mentioned earlier, information is not available regarding the exact structure of the RAR endings. It could be speculated that the RAR in the lungs and tracheobronchial tree may also be surrounded by connective tissue that somehow diminishes a constant effective stimulus reaching the nerve terminal with time. Whether this is the case or whether the excitable membrane of the RAR ending possesses characteristic properties different from that of the SAR is a matter of conjecture.

Another point worth considering is whether a maintained lung inflation is in fact the appropriate stimulus for testing the rate of adaptation of the RAR. Historically, RAR had been studied together with SAR and by implication both types of receptors have been regarded as mechanoreceptors. While this is undoubtedly the case with SAR, the same may not necessarily be true for the RAR. Consider the large number of names given to the RAR in the past, each indicating a function believed to be subserved by the receptor. Perhaps the changes during lung inflation brings a secondary stimulus which in turn stimulates the RAR. Then, as the lung inflation is maintained,

the secondary stimulus may no longer be operative and thus the RAR is not stimulated. Hence the appearance of rapid adaptation. If this is in fact the case, then when an "appropriate natural stimulus" is applied, the RAR may not be rapidly adapting. The search for this "natural stimulus" continues.

At normal respiratory rate and tidal volume, it is thought that RAR are relatively inactive (114,116), one study reported frequencies of 0.2-0.3 impulses/sec in the dog (116). With increases in tidal volume or airflow or both, RAR activity increases (119,120). This was demonstrated in vagotomised rabbits (120), in which the increase in RAR activity was thought to be brought about by the increased depth of inspiration. Pack and Delaney showed that, with successive lung inflations at increasing rates of flow and within the physiological range, the firing threshold of the RAR decreased and impulse frequency at each volume increment increased (115). This has been interpreted that the RAR provided a signal related to the degree of inflation. However this response is much less sensitive and more variable than that of the SAR (119).

Deflation usually also stimulates RAR (55,56,104,116,120,121). The RAR showed an increase in activity which is irregular. When the lungs are collapsed, the receptors may discharge with a cardiac modulation, which is thought to be due to the high degree of mechanical sensitivity of this group of receptors to the pulsations of the heart and/or nearby blood vessels (55).

Responses of RAR to Other Stimuli

The most important function of RAR is said to signal the onset of pathophysiological changes in the airways (56,101,102,103,120). The

responses discussed below are considered with this function in mind.

(1) Pulmonary Congestion and Oedema

There are a few reports in the literature describing the responses of the RAR to increments in pulmonary venous pressure and their findings raise several issues which form part of the series of investigations carried out in the present study. Specifically, it is found that the intensity of the stimulus applied, i.e., the increase in pulmonary venous pressure, was usually in excess of changes observed in common physiological or pathophysiological settings (121), and that the stimulus was usually applied for a relatively short period of time (usually 1-2 minutes) (120,121).

RAR were found to be activated consistently by pulmonary congestion. There was, however, no obvious correlation in the changes of RAR activity with changes in total lung resistance or compliance (120,122,123). With the onset of pulmonary oedema following congestion, RAR were activated markedly (91,120). The mechanism of activation of RAR during pulmonary congestion and oedema is not known and it has been suggested that this may be due to the activation of RAR endings by a local increase in blood pressure, local contraction of smooth muscle, and/or the release of chemicals that may directly stimulate the receptors, or indirectly by causing changes in lung mechanics (120,121). Another possible mechanism for the excitation of the RAR may be that the nerve endings are activated by the fluid accumulating in the interstitial spaces during pulmonary oedema (124).

A recent investigation of the effects of pulmonary congestion and oedema induced by massive fluid infusion showed that the RAR were

activated consistently with the onset of pulmonary congestion. It is of interest to note that the RAR activity decreased to control levels after relief of the congestion, but leaving behind lung oedema (37). Mechanisms related to lung mechanics that are operative during pulmonary congestion but not present during passive lung oedema have been suggested to be the stimuli for activating the RAR though the precise nature of the mechanisms remains unclear.

(2) Pulmonary Microembolism

Like SAR, RAR are activated by pulmonary microembolism (90,93). It is unclear whether RAR were activated by a decrease in lung compliance and pulmonary obstruction (90,93) or by local mechanical alterations possibly mediated by the release of some active substances (92,93)

(3) Anaphylaxis

During induction of anaphylactic shock, RAR activity was found to be increased markedly in the guinea pig and rabbit (75,93). The increase in activity did not correlate with the observed changes in total lung resistance and compliance (93). Mechanisms such as local mechanical changes due to smooth muscle contraction or the release of active substances which may have activated the receptor endings, or uneven ventilation of the lungs have been suggested (193).

(4) Inhaled Irritants

RAR are stimulated by many inhaled substances, aerosols or fumes such as ammonia (102,114,123), ethyl ether (114), cigarette smoke and inert dusts (103). In one study, in cats, sulphur dioxide sensitised one third of RAR but had little effect on the rest (57). Other studies showed that high concentrations of sulphur dioxide had little

effects on RAR in rabbits (100) and dogs (125). There appear to be differences in response among species. It has been found that RAR from rabbits showed greater responses to ammonia and cigarette smoke than RAR from dogs (103,116). The mechanism of activation of RAR by these substances has been suggested to be due to changes in lung mechanics brought about through the release of some active substances (40).

(5) Other Substances which Influence Bronchomotor Tone

Activation of RAR by histamine has been attributed to an indirect effect acting via contraction of the airway smooth muscle as well as a direct effect on the receptor endings (76,105,126,127). A secondary effect of histamine on the receptor endings due to contraction of the airway smooth muscle was found in guinea pigs and rabbits. When the airway smooth muscle was relaxed by isoproterenol, this prevented the changes in lung mechanics induced by histamine. RAR activity also did not increase (128). This finding was disputed by Coleridge et al (129) who suggested that the effect of histamine on the RAR was to sensitise them to the effects of the intervening bronchoconstriction.

Acetylcholine is believed to activate RAR by an indirect effect through a change in lung mechanics (109,119,122,126,127). Serotonin is thought to have both a direct and indirect effect on the receptor endings (115).

The prostaglandin $F_{2\alpha}$, given as an aerosol, has been shown to activate the RAR, though the evidence for either a direct or indirect mode of action remains inconclusive (127,129,130).

Bradykinin has a weak stimulating effect on RAR and it has been suggested that it acts by sensitising the receptor to mechanical

changes induced in the lung itself (130,131).

Phenyldiguanide and capsaicin, two chemicals commonly used to evoke the pulmonary chemoreflex, increase the activity of RAR in rabbits (101) and cats (123). Veratrum alkaloids also stimulate RAR (40,132)

(6) Carbon Dioxide

An increase in alveolar CO_2 does not seem to activate RAR but a marked increase in RAR activity in the artificially ventilated dogs occurred when airway CO_2 was diminished. This activity was inhibited when $[CO_2]$ was returned to normal (97). In spontaneously breathing rabbits, an increase in inspired CO_2 has been shown to increase RAR discharge and augment ventilation (120). The increased activation of RAR is believed to be due to the increased ventilation (tidal volume and rate) associated with the stimulating effects of CO_2 on respiration. The role, if any, played by RAR on the "pulmonary CO_2 reflex" remains to be evaluated.

C-fibre Receptors

Evidence from studies conducted in the 1950's and early 1960's indicated the presence of a group of receptors originating from the lungs and tracheobronchial tree that discharged into non-myelinated fibres of the vagi (58,59,60,99,128,133,134,135). These receptors are referred to here as "C-fibre receptors" and they are thought to respond to stimuli such as pulmonary inflammation, congestion, embolism and other pathophysiological conditions which could be potentially harmful (136).

C-fibre afferents have been grouped into two distinct groups:

pulmonary C-fibre receptors and bronchial C-fibre receptors (60). This classification was based on the responses of the C-fibre receptors to a number of stimuli, such as to lung inflation, but principally to the injection of phenyldiguanide and capsaicin into the right atrium. Pulmonary C-fibre receptors are thought to be found in more peripheral areas of the lungs while bronchial C-fibre receptors are found in the larger bronchioles, bronchi and lower trachea. This distinction was made on the different blood supply these receptors are thought to have. The pulmonary C-fibre receptors are perfused by blood from the pulmonary circulation while bronchial C-fibre receptors receive their blood supply from the bronchial arteries. Receptor location made on such a basis has been criticized by Sant'Ambrogio who, working with SAR and RAR, pointed out the inherent inconsistency of the pulmonary and bronchial circulations in supplying the lungs and larger airways respectively and cautioned that such classification may not be accurate if these findings were to be extrapolated to C-fibre receptors (40,132). However, this classification, which is accepted generally, appears convenient enough in indicating the general location of these receptors. The proportion of C-fibres subserving pulmonary and bronchial receptors is not known.

These pulmonary C-fibre receptors do not appear to form a homogeneous group. They have been known by various names: J-receptors (59,135), deflation receptors (58,133), high threshold inflation receptors (99,134) and alveolar nociceptors (128), indicating the various stimuli which could activate some of the receptors. Conduction velocities (<2.0 m/s) and differential vagal blockade

indicate that these receptors discharge into non-myelinated vagal fibres (63,64).

There appears to be no general acceptance of the role played by these afferents in the physiological regulation of respiration. Also, while the evidence for a "protective" function appears clearer, the part played by these receptors in the "protective" reflexes need further evaluation.

Experiments involving vagotomy above the nodose ganglion causing degeneration of efferent fibres and then counting the remaining afferent fibres have been carried out. The results showed that in the vagus nerve of the cat, the ratio of non-myelinated to myelinated afferent fibres was approximately 3-4:1 (approximately 3800 non-myelinated fibres) (137), a proportion that is similar to that found in cutaneous sensory nerves (138). Another study, also in cats, suggested an even higher ratio of 9:1 (139).

Morphology of C-fibre Receptors

Little is known of the morphology of C-fibre receptors. It is of interest to note Paintal's suggested schematic representation of a typical pulmonary C-fibre receptor ending lying in the interstitial tissue between a capillary and an alveolus surrrounded by collagen fibres (59,135,140). The afferent nerve ending is thought to be stimulated by an increase in interstitial volume when the inflow of fluid into the interstitial tissue is greater than the fluid removed. The excitation is effected at the "generator" region of the ending. It is also suggested that excitation by chemical substances from outside through alveolar air, or inside through the blood occurs by an action on a "regenerative" region. This hypothetical pulmonary

C-fibre ending has not been demonstrated definitely as it requires both electrophysiological studies and anatomical description of the same ending. However, some histological description of afferent nerve endings adjacent to alveoli have been reported. The evidence for the presence of pulmonary C-fibre receptors is summarised below.

Unlike the SAR and RAR, it is not known whether the termination of the axon of the C-fibre receptor shows branches and arborizations. The relation of the surrounding structures, and the density of the sensory fields remain unclear. Light and electron microscopy studies identifying afferent endings are made based on what is known of sensory endings, i.e., the presence of a terminal axonal enlargement with large numbers of mitochondria, partially sheathed in a Schwann cell and with close contact between the axonal membrane and some adjacent cells (69,76,137,141,142,143). These studies were usually done following "degeneration" experiments to eliminate efferent endings by a vagotomy above the nodose ganglion.

Studies have been made of these endings in the mouse and rat lungs and from samples of human lungs (141,142,143). Most studies have demonstrated a scarcity of nerve endings in the alveoli. For example, Meyrick and Reid (141) found non-myelinated fibres with features indicating a sensory function in the alveolar walls in 2 of 80 small blocks of lung tissue in 40 rats. Fox et al (143) found non-myelinated fibres in the alveolar walls in 3 of 50 blocks taken from samples immediately beneath the pleura in 16 human lungs. There is no certainty that these nerve elements are the C-fibre endings. However, it is suggested that these endings, usually associated with type I pneumocytes in the alveolar walls, are equivalent to the

J-receptors described by Paintal in electrophysiological studies. Such an ending has been described as characteristically showing an axon or bundle of axons in the interstitial tissue surrounded by collagen fibres (141,142,143).

In the proximal airways, non-myelinated afferent fibres have been described as naked nerve endings found between the epithelial cells of the airway mucosa (144). In human and mouse lungs, these bundles of non-myelinated fibres have been found in the lamina propria and some axons were seen to penetrate the basement membrane and then give intra-epithelial endings (144). It has been suggested that these axon endings may correspond to the bronchial C-fibre receptors (144).

Patterns of Discharge of C-fibre Receptors

Unlike the SAR and RAR, pulmonary and bronchial C-fibre receptors do not show a temporal relationship to respiratory movements. Instead, they discharge irregularly and at a low frequency during eupnoea (60,145). When pulmonary C-fibre receptors were originally studied, they were called "deflation receptors" because of their stimulation during deflation of the lungs though this response was later not found to be consistent (58,133).

Large lung inflation, by 2 or more tidal volumes, also elicits a response in some of these pulmonary C-fibre receptors in cats and dogs (60,99,124,134). Bronchial C-fibre receptors are thought to have a smaller response to lung inflation, some are said not to be affected even by hyperinflation (60,146).

Whether the afferent activity studied from experimental animals was recorded with an open or closed chest has an influence on the pattern and frequency of discharge. It has been reported that in

artificially ventilated dogs and cats with open chest, the average discharge frequencies were 0.4 impulses/s for pulmonary C-fibre receptors and 0.8 impulses/s for bronchial C-fibre receptors (60,145). During spontaneous breathing, the discharge frequency increased significantly to 1.9 impulses/s in pulmonary C-fibre receptors (145) but bronchial C-fibre receptors remained unchanged (60).

These receptors are identified by their characteristic response to administration of phenyldiguanide and capsaicin into the right atrium (see pg 27 re classification of C-fibre receptors). Thus, pulmonary C-fibre receptors are stimulated around 2s after injection of these chemicals into the right atrium and the receptors respond with a burst of high frequency discharge activity (60).

This short latency for stimulation has been suggested to indicate that the pulmonary C-fibre receptors are accessible to blood from the pulmonary artery (60). In the case of the bronchial C-fibre receptors, the latency time is longer, usually 7 s or more, which suggests that these receptors are stimulated only when these chemicals have reached the bronchial arteries (60). When chemicals were injected into the left atrium or a bronchial artery, the latency time was much reduced (4-8 s) (60,131). The bronchial C-fibre receptors respond with a similar burst of high frequency activity (60,131).

Coleridge and associates have reported that, in the dog, unlike the cat and rabbit, the majority of pulmonary C-fibre receptors are not affected by phenyldiguanide even though an occasional receptor may be so stimulated (60,134).

Responses of C-fibre Receptors to Other Stimuli

1. Foreign Chemicals

Pulmonary C-fibre receptors in dogs and cats are stimulated by high concentrations of halothane, ether, trichloroethylene and chloroform (99). They are also stimulated by alloxan (59,145), chlorine (59), and sulphur dioxide (126). Bronchial C-fibre receptors are stimulated by sulphur dioxide (125) but the response of bronchial C-fibre receptors to alloxan or chlorine has not been studied.

2. Lung Autocoids

Serotonin stimulates bronchial C-fibre receptors in cats, dogs and rats but do not stimulate the pulmonary C-fibre receptors in dogs (131). This stimulation appears to be due to a direct effect and not secondary to the stimulation of the airway smooth muscles.

Prostaglandins, particularly of the E series, stimulate pulmonary C-fibre receptors (131).

Bronchial C-fibre receptors have been found to demonstrate a higher chemosensitivity than pulmonary C-fibre receptors (60,129,130, 131,147,148). In dogs, bronchial C-fibre receptors are stimulated by small doses of prostaglandins, PGE_2 and PGI_2 (129,130), histamine (131,147,148), and bradykinin (131), injected either into the left atrium or bronchial artery, or administered as an aerosol into the airways and lungs.

3. Carbon Dioxide

Pulmonary C-fibre receptors have been thought to play a role in the "pulmonary CO_2 reflex" (149,150). It has been shown in one study that the activity of pulmonary C-fibre receptors doubled from 1 impulse/s at a pCO_2 of 14 mm Hg to 2 impulses/s at a pCO_2 of 70

mm Hg. However, at a pCO_2 above 28 mm Hg, the majority of these receptors studied failed to increase their activity and all the receptors adapted rapidly at any given level of pCO_2 (149,150). Thus, the role of pulmonary C-fibre receptors in this reflex does not appear clear at all.

4. Pulmonary Congestion and Oedema

Paintal suggested that the natural stimulus for pulmonary C-fibre receptors is pulmonary congestion (59,135). He postulated that these receptors are found in the interstitial tissue surrounded by collagen fibres which would swell, in the presence of increased interstitial fluid during pulmonary congestion or oedema, become distorted and stimulate the receptor endings.

Some of Paintal's earlier work on the effect of pulmonary oedema on pulmonary C-fibre receptors involved the production of pulmonary oedema by the intravenous administration of alloxan or inhalation of chlorine gas (58,60). The mechanism of action of both alloxan and chlorine is believed to be due to an increased permeability of the pulmonary capillaries leading to interstitial oedema, followed in a relatively short time by production of alveolar oedema as demonstrated by the presence of oedema fluid in the airways. The conclusion drawn was that the presence of oedema in the interstitium stimulated these receptor endings (58,59,135). However, there is the possibility that the chemicals used for producing pulmonary oedema might have stimulated these receptors directly.

In a later investigation, Paintal studied the effect on the pulmonary C-fibre receptor activity in the cat by increasing blood flow to one lung with occlusion of the pulmonary artery to the other

lung (151). During this manoeuvre, it was found that activity of the pulmonary C-fibre receptors in the lung with the augmented blood flow increased significantly though not all the receptors responded in this way (151). A recent study by Coleridge's group showed that pulmonary oedema produced by a relatively massive infusion of crystalloid fluid in dogs stimulated these pulmonary C-fibre receptors. These receptors remained activated when the lung microvascular pressure was restored to control levels by withdrawal of blood but with the increased lung water remaining unchanged (37).

These studies provide the evidence that the pulmonary C-fibre receptors are activated by "pulmonary oedema". However, the evidence is less clear concerning the activity of the pulmonary C-fibre receptors in the presence of pulmonary interstitial congestion without gross alveolar oedema.

A few other studies have shown also that bronchial C-fibre receptors are stimulated by pulmonary congestion and oedema (37,145). One recent study demonstrated that the bronchial C-fibre receptors were activated to some extent when there was severe lung oedema accompanied by peribronchial cuffing and alveolar oedema (37).

5. Pulmonary Embolism

Both pulmonary and bronchial C-fibre receptors are stimulated by pulmonary embolism (92). The mechanism of stimulation appears complex and could be due to a combination of mechanical distortion of small pulmonary vessels and release of chemical mediators such as histamine which may have a direct effect on the receptors, or via a secondary action by causing contraction of the airway smooth muscles (92,135).

6. Inflammation

Like pulmonary embolism, both pulmonary C-fibre and bronchial C-fibre receptors are stimulated during lung inflammation (152). The mechanism of stimulation appears to be similar to that discussed above for pulmonary embolism (92,153).

Summary of Studies on Pulmonary Receptors

From these studies, the following summary can be drawn :

SAR

(1) SAR discharge into myelinated afferent fibres of the vagus nerves.

(2) The morphology and location of the SAR have been described. SAR are thought to form terminal branches which lie between the lamina propria and smooth muscle layers of the tracheobronchial tree. The branch endings are believed to be attached to the underlying smooth muscle by bands of elastin and collagen, and are orientated in the long axis of the airways.

(3) SAR form the afferent link of the Hering Breuer inflation reflex and it has been suggested that they play a role in regulating the duration both of inspiration and expiration during eupnoea.

(4) The responses of SAR to pulmonary congestion, oedema, micro-embolism and atelectasis appear to be secondary to the changes in lung mechanics associated with these pathological conditions.

(5) The role, if any, of SAR in other regulatory and defensive reflexes arising from the tracheobronchial tree needs further investigation.

RAR

(1) RAR discharge into myelinated afferent fibres of the vagi.

(2) RAR have been shown to respond to irritant stimuli introduced into the lungs and tracheobronchial tree.

(3) RAR have been shown also to respond to inflation and deflation with rapid adaptation.

(4) The morphology of the RAR terminal has been suggested to consist of terminal arborizations which are distributed in different layers of the airway wall, with some terminal branches lying in between the epithelial cells while others lie deeper in the muscle layers.

(5) RAR are thought to play a role in the defensive reflexes associated with the introduction of noxious stimuli into the lungs and airways as well as during pulmonary congestion, oedema, microembolism and atelectasis. However, the evidence for these remains unclear and the mechanism of stimulation of these receptors remains to be evaluated.

C-fibre Receptors

(1) These receptors discharge into non-myelinated fibres of the vagi.

(2) Not much is known of the location, structure and morphology of these receptors. A hypothetical description of a pulmonary C-fibre receptor has been described of a structure lying in the interstitial tissue between a capillary and an alveolus surrounded by collagen fibres.

(3) There appear to be two groups of C-fibre receptors, classified according to their blood supply from the pulmonary circulation (pulmonary C-fibre receptors) and bronchial arteries (bronchial C-fibre receptors).

(4) A role in the defence reflexes arising from the lungs and

tracheobronchial tree has been suggested for these receptors.

General Comments - Cautions in the Interpretation of Data

(1) Studies on the receptors have been performed on different animal species. The existence of species differences is always a possibility and has to be borne in mind in interpreting the results.

(2) A number of reflexes were studied using chemicals which are not found in the normal physiological and pathophysiological situations. The relevance of these findings needs further evaluation.

(3) In extrapolating from electrophysiological studies in animals to possible control mechanisms, the experimental protocols used have to be evaluated carefully. Issues such as the physiological relevance of the stimuli, and the duration of their application are of particular importance.

Thus, caution should be taken in extrapolating results obtained from a different animal species, particularly when the experimental conditions differ.

CHAPTER 5

REVIEW OF CARDIAC RECEPTORS

Atrial Receptors

Afferent nerve endings are found within the walls of the atria, the atrial appendages, at the junctions of the right atrium with the superior and inferior vena cavae and of the left atrium with the pulmonary veins. The majority of receptors are complex unencapsulated endings with some existing as free nerve endings (154,155). These atrial receptors have been extensively studied and a number of reflexes have been reported to be caused by stimulation of these receptors. Their stimulation causes (i) a cardiac chronotropic response without an inotropic effect (156) and (ii) a renal response consisting of a hypo-osmolar diuresis and natriuresis (157). The effect of stimulating atrial receptors on the lungs and tracheobronchial tree is not known.

The majority of atrial receptors discharge into myelinated fibres that form branches of the vagus nerves (158). However, atrial receptors discharging into non-myelinated fibre receptors have also been demonstrated (158). The following review deals with atrial receptors discharging into myelinated fibres. Like pulmonary receptors, some of the atrial receptors also discharge into sympathetic afferent fibres (159).

Patterns of Discharge

In 1953, Paintal suggested that atrial receptors could be divided into two types, A and B (160,161). Type A receptors discharged during atrial systole and coincided with the 'a' wave of the atrial pressure wave. Type B receptors discharged during atrial diastole, coinciding

with the 'v' wave. This classification suggested differences in the receptor types, one responding to changes in atrial contractility and the other to changes in atrial volume. This classification was challenged by Coleridge et al (154) and by Henry and Pearce in 1956 (162). Both groups of investigators converted classical type B discharge to type A discharge by bleeding the animals and then reversed the process by retransfusing the blood.

It is now generally accepted that the pattern of discharge depends on the receptor location (158). Under normal conditions, type A discharges are usually demonstrated by receptors located near the pulmonary vein-atrial junctions where there is greatest distortion during atrial contraction whereas type B discharges are shown by receptors located in the lateral atrial walls or in the distal pulmonary veins which are more responsive to volume changes (163). By changing the atrial geometry, the forces acting on the atrial receptors during the cardiac cycle could be altered and thus change the patterns of discharge (158).

Experimental Methods of Stimulation of Atrial Receptors

Atrial receptors can be stimulated by stretching of the atrial wall in which they are located. Small balloons at the end of fine cannulae can be inserted into the pulmonary veins so that the balloons lie at the pulmonary vein-left atrial junctions. Inflation of the balloons causes localised stretching of the atrial wall and stimulation of the atrial receptors located there. A chronotropic response without changes in arterial pressure is noted when the atrial receptors are stimulated (156,164). It has been found that the degree of reflex tachycardia could be graded by varying the number of

pulmonary vein-left atrial junctions that are stretched. In one study in the dog, it was found that an average increase in heart rate of 10.8 beats/min was found with stretching one junction, 22.2 beats/min with two junctions and 35.2 beats/min with three junctions (165). However, it was not possible to grade the amount of stimulation at a single site by varying the distending pressure. One may speculate in this case that the receptors were stimulated by an "all or none" mechanism.

A balloon at the end of another cannula can be introduced into the left or right atrial appendage so that inflation of these balloons causes partial obstruction of the mitral and tricuspid valves, respectively. In each case, drainage of blood from the atrium into the ventricle was impeded, the atrial pressure increased, the atrial wall became stretched and the atrial receptors were stimulated (166,167,168) as shown by the reflex increase in heart rate.

Another way of stimulating the receptors at the superior vena cava-right atrial junction has been described (168). An expansile metal cannula was introduced into this junction via the right external jugular vein. The metal cannula could be expanded without impeding venous return into the right atrium. The superior vena cava-right atrial junction was stretched and the right atrial receptors at this site were stimulated.

The receptors at the pulmonary vein-left atrial junctions could be stimulated by stretching these junctions "externally" (of the heart) by applying sutures to each junction. When tension was applied to the sutures in different directions, the junction became stretched and the atrial receptors were stimulated (169).

Physiological Roles of Atrial Receptors

The chronotropic response due to stimulation of atrial receptors has been well documented (156,158,166,167,168). The afferent limb of the reflex is formed by afferent fibres in the cervical vagus nerves while the efferent limb is largely sympathetic in origin, travelling in the right ansa subclavia (164,170). In 1915, Bainbridge showed an increase in heart rate following infusion of fluids into the heart in anaesthetized dogs (171). This reflex effect (Bainbridge effect) was suggested to be mainly due to the withdrawal of vagal tone and was considered to be one of the control mechanisms which prevented over-distension of the heart (171). This original study was followed by others, some of which showed a similar effect (172), while others either failed to show any effect or a divergent effect (173). It appears that the role of the initial heart rate had a major influence on the response, a relatively slow heart rate responded with an increase whereas one that was fast initially may fail to increase further, or even decrease. For example, one study showed that reflex tachycardia occurred with stretching of the pulmonary vein-left atrial junction when the initial heart rate was <140-150 beats/min but slowing of heart rate occurred with a faster initial heart rate (174).

Stimulation of atrial receptors has no apparent effect on respiration (175) or peripheral resistance (176). Some investigators have shown a transient peripheral vasodilatation which they attribute to stimulation of non-myelinated fibre receptors (174).

Vasodilatation in the kidneys occurred following stimulation of the atrial receptors, due to a decrease in renal sympathetic

activity, an effect not found in the other sympathetic nerves (170). Urine flow increased concomitantly (157,162). This effect has been suggested to be partly due to an alteration in renal blood flow and a change in urine composition (157,162). The increase in urine flow was considered to be due to the inhibition of anti-diuretic hormone thus resulting in a diuresis (177). However, results of experiments in which the effects or the presence of anti-diuretic hormone were abolished could not explain satisfactorily the change in urine flow and composition (178). Another hormone, called atrial natriuretic factor, has been isolated from blood and found to be secreted from atrial tissue (179,180,181). This hormone is now shown to be responsible for the diuresis when the atrial walls are stretched.

Summary on Atrial Receptors

From these studies the following conclusions can be drawn :

(1) Most of the atrial receptors consist of complex non-capsulated endings discharging into myelinated fibres of the vagus nerves.

(2) Atrial receptors are stimulated by stretching of the atrial wall, the pattern of discharge depending on the location of the receptors and the forces acting locally on the receptors.

(3) Physiological reflexes associated with stimulation of atrial receptors are a chronotropic cardiac response and a reflex increase in urine flow. This latter effect is partly also mediated by atrial natriuretic factor, a hormone produced from the atrial wall.

(4) The effects of stimulation of the atrial receptors on the lungs and tracheobronchial tree are not known.

Cardiac C-fibre Receptors

Unlike the myelinated atrial afferent nerve endings discussed in the preceding pages, the cardiac non-myelinated C-fibre receptors have been less extensively studied. However, it is known that activation of these receptors brings about a number of reflexes, some of which are similar to those associated with C-fibre receptors of the lungs (182,183,184,185,186).

Atrial C-fibre Receptors

Like lung C-fibre receptors, C-fibres in the atria have a very low resting discharge frequency (usually < 1 impulse/s). They may show an increase in activity during the inspiratory and early expiratory phases of the respiratory cycle (176) and therefore may be mistaken for lung receptors. This phasic change during respiration is thought to be due to the changes in blood volume and to the changes in transmural atrial pressure during respiration (182). The function of these atrial C-fibre receptors remains unclear.

Ventricular C-fibre Receptors

In the dog, it has been found that injection of small amounts of veratridine or nicotine into the left (but not the right) coronary artery stimulated sensory endings which cause reflex bradycardia and hypotension (183). These reflex changes can also be evoked from the ventricular receptors by applying small pieces of filter paper soaked in nicotine or acetylstrophanthidin onto the left ventricle (184,185). The reflex has been called the coronary chemoreflex or Bezold-Jarisch reflex (186).

Some of these ventricular C-fibre receptors are activated by capsaicin and phenyldiguanide (like lung C-fibre receptors) while

others are stimulated by occlusion of the aorta, i.e., they function as mechanoreceptors responding to the changes in ventricular pressure (187). These mechanoreceptors are also activated during systole with the increase in myocardial tension, increase in diastolic volume during blood transfusion or by interventions causing a positive inotropic effect (188,189). The activity of some ventricular C-fibre receptors increases during asphyxia (187,190). The mechanism of this is believed to be due not to the changes in arterial pO_2 or pCO_2, but rather to the mechanical dilatation of the ventricles (190). The common response to the stimulation of these receptors is a reflex bradycardia and a peripheral vasodilatation due to a reduction in the sympathetic adrenergic-mediated vasoconstriction (191,192).

The Bezold-Jarisch reflex has also been shown to be blocked or attenuated by the intravenous injection of local anaesthetic drugs. This is thought to be due to "endoanaesthesia" of the afferent C-fibre endings (193). Thoren reported such an effect when using a dose of 3-4 mg per kg body wt of lidocaine to achieve a plasma level of 2.5 to 4.7 ug lidocaine per ml plasma (193).

It has been suggested that the Bezold-Jarisch reflex may be of importance both for normal cardiovascular control and during common pathophysiological conditions such as fainting (190), aortic stenosis (194), myocardial infarction (193) and asphyxia (187,190).

Right Ventricular C-fibre Receptors

In the right ventricle of the dog, C-fibre receptors are believed to be less numerous than receptors discharging into myelinated fibres (184). As a result, right ventricular distension brings about weak reflex responses or none at all (189).

Electrophysiological Studies

Like lung C-fibre receptors, the ventricular C-fibre receptors are mostly identified on the basis of electrophysiological studies (e.g. 184,187,189). No histological description of these endings has been reported. Histological studies of the cardiac vagal branches of the cat showed that there are about 2000-2500 afferent fibres of which about 75% are C-fibres (137). There is some evidence to suggest that these receptors may be situated in the superficial layers of the epicardial surfaces of the ventricle (187,195) with others scattered through the ventricular wall (196).

Summary on Cardiac C-fibre Receptors

(1) Afferent endings discharging into non-myelinated fibres of the vagi are present both in the atria and ventricles.

(2) While the function of the atrial C-fibre receptors are unknown, the C-fibre receptors in the left ventricle, when activated, evoke the Bezold-Jarisch reflex, a depressor reflex consisting of bradycardia and hypotension. This reflex is not unlike that initiated by the C-fibre receptors of the respiratory system. C-fibre receptors in the right ventricle are less numerous and the reflex effects on activation of these receptors are weak.

(3) The structure and morphology of these receptors, which are identified on the basis of electrophysiological studies, is not known.

CHAPTER 6

REVIEW OF TRACHEOBRONCHIAL SMOOTH MUSCLE

The calibre of the airways depends on the tone of the smooth muscle of the tracheobronchial tree. The importance of this tone is well illustrated in bronchial asthma. While different mechanisms are thought to be involved in cardiac asthma, a possible reflex bronchoconstriction during pulmonary venous congestion cannot be excluded. It is not intended here to review extensively the subject of airway luminal diameter changes. Instead, it is planned to focus attention mainly on the following :

A. Methods of studying tracheobronchial muscle tone,

B. Anatomical and physiological considerations,

C. Reflex regulation of tracheobronchial smooth muscle tone,

D. Effects of various chemicals,

E. Implications in asthma - bronchial and cardiac.

A. Methods of Studying Tracheobronchial Muscle Tone

In order to study the properties of airway smooth muscle, a number of techniques are being used to measure the smooth muscle tone. Each method has its own advantages and drawbacks. A number of commonly used methods are discussed here.

1. Study of Smooth Muscle Tone In-vitro

This method is suitable in studying the effects of certain drugs on the smooth muscle, e.g., the effect of adrenergic agonists and antagonists on a preparation of tracheal smooth muscle which may or may not be pre-contracted by another agent such as methacholine (197). While the method allows accurate and

continuous measurement of smooth muscle tone, it obviously cannot take into account the modulating effects of the action of the efferent nerves and biochemical and hormonal environment found in the intact animal.

2. Measurement of Airway Function (Compliance and Resistance; Closing Volumes)

These methods monitor changes in airway flows as indirect measurements of tracheobronchomotor tone (6,198). Continuous or frequent accurate monitoring can be performed. The drawback with these methods is that they are indirect and the changes noted may be due to other influences other than changes in tracheobronchomotor tone.

3. Measurement of Airway Diameters by Serial Tantalum Bronchograms

This method has been used successfully to monitor changes in airway smooth muscle tone indirectly and has the advantage that it can be repeated as required (5,199). However, it suffers from some disadvantages: (i) it is indirect, (ii) care is required to measure the same bronchi in serial studies, and, (iii) the development of mucosal oedema may be measured as increases in smooth muscle tone.

4. Recording Isometric Contraction in the Trachealis Muscle of an Innervated Segment of the Upper Trachea

This method was described by Brown et al in 1980 (200). It involves continuous recording of the isometric tension of the trachealis muscle that remains innervated in an upper tracheal segment in the intact animal. Thus, changes in tension can be monitored directly in an easily accessible part of the airways

and it allows reflex bronchomotor mechanisms to be studied. By leaving the motor innervation to the segment from the superior laryngeal nerves intact and interrupting the recurrent and pararecurrent laryngeal nerves, examination of the effects of blocking the afferent pathway in the cervical vagus nerves is possible without interfering with the vagal efferent pathway to the smooth muscle of the tracheal segment under study. The drawback is that one can never be sure if changes in the tracheal segment bear any resemblance to changes occurring in the lower airways. It has been suggested, however, that this may be more of a theoretical than practical problem (39). The reasons for this are: (i) the trachealis smooth muscle has ultrastructural, mechanical and pharmacological properties similar to those of the smooth muscle in the large bronchi (201), (ii) the tracheal and bronchial smooth muscles have similar length-tension characteristics in vitro (39), and (iii) a similar time course was observed in the changes in airway smooth muscle tone in studies on airway reflexes following the use of capsaicin, using serial tantalum bronchograms (199) and the above technique (202). It was concluded that reflex changes in tracheal tone appear to provide a good indication of the direction of changes in tone of the lower airways (39).

Measurement of the isometric contraction of an innervated upper tracheal segment will be used in the present workis and further details of the method will be described under Experimental Methods (see pg.144-145).

B. Anatomical and Physiological Considerations

In the trachea of the dog and other mammals, the effective muscle is the posterior trachealis muscle. Most of the fibres of this muscle run transversely and are chiefly responsible for the active changes in tracheal volume (203). There are also some oblique and longitudinal fibres, which, in addition to the interrosei muscles between the cartilages, slightly shorten the trachea during tracheal muscle contraction.

The alignment of the muscle fibres in the bronchi, which have cartilaginous plaques around the luminal wall, are more oblique. In the bronchioles and alveolar ducts, the smooth muscle has been described to be thicker relative to the luminal diameter than in the larger airways (203). However, it is not known whether, in these smaller airways, the relatively greater bulk of muscle and the relatively small airway diameters would have a disproportionately greater influence on the airway diameters (according to Laplace's Law) and the airway resistance as a whole. Indeed, it has been suggested that these smaller airways have distensibilities similar to the alveoli (204). Further, the trachea and lobar bronchi have been reported to have a distensibility of similar magnitude, suggesting that the lung and air passages increase their volume approximately proportional to the changes in transpulmonary pressure (205).

It has been shown, by pressure/volume measurements, that a maintained transmural pressure difference of 10 cm H_2O can increase the volume of the trachea by 40% in the dog (206). In the bronchi, a similar or greater distensibility (of up to twice that of the trachea) has been shown despite the presence of the encircling

cartilages. Also, bronchial diameter has been shown to decrease by up to 70% in response to bronchoconstrictor drugs and increase by 25% by bronchodilators (203). Like the lungs, both trachea and bronchi also show hysteresis and stress relaxation (206,207).

Innervation

The sensory afferent innervation of the tracheobronchial tree has been reviewed in the section on receptors of the lungs and airways. The efferent innervation of the airway smooth muscle is via the parasympathetic and sympathetic nerves. In general, the vagal parasympathetic nerves are excitatory to the airway smooth muscle and cause bronchoconstriction while sympathetic activity results in bronchodilatation (39,201,208). However, there is some controversy as to the role of the sympathetic innervation which is known to vary with the species studied (208). For example, the density in adrenergic innervation have been found to be higher in cats, dogs and calves than in rabbits, rats and guinea pigs (209,210,211). In cats and dogs, sympathetic innervation has been demonstrated as far distally as the respiratory bronchioles while in rabbits the evidence for an adrenergic nerve supply to the airway smooth muscle remains unclear (211). Under normal conditions, a small degree of airway smooth muscle tone appears to be present due to a tonic low frequency activity of the vagal bronchomotor fibres. This tone could be reversed by cervical vagotomy, vagal cooling, or atropine administration (208).

Efferent vagal innervation of the trachea is via fibres from the superior laryngeal nerves to a variable upper segment and the rest by fibres from the recurrent and pararecurrent laryngeal nerves. The

innervation of the rest of the airways is from pulmonary branches the vagi. It has been suggested that nervous regulation affects mainly the smooth muscle of the larger cartilage-bearing airways (39).

C. Reflex Regulation of Airway Smooth Muscle Tone

A large number of stimuli, not all of which are included in the following list, are known to affect the tracheobronchomotor tone. While it is clear that an increase in tone is due to a parasympathetic excitatory effect, the same cannot be said of the reduction in tone. The latter may be due to an inhibitory effect on the parasympathetic maintained tone or an enhancement of sympathetic activity. The great majority of studies did not address this question. However, it can be speculated that, as a result of the effect of the afferent input on the medullary centres controlling airway smooth muscle tone, an inhibitory effect on the parasympathetic-maintained tone may exist.

1. Inputs from Upper Respiratory Tract

It has been shown that mechanical and chemical stimuli to the nose and larynx cause bronchoconstriction (212,213). However, stimulation of the nasopharynx has been shown to be associated with reflex bronchodilatation (213)

2. Input from Lower Airways

Slowly adapting stretch receptors are reported to have a tonic inhibitory influence on airway smooth muscle (214), while rapidly adapting receptors have a bronchoconstricting effect (93). Receptors in the lungs and airways discharging into non-myelinated afferent fibres, i.e., pulmonary C-fibre and bronchial C-fibre receptors, are also thought to have a bronchoconstricting effect (39,202). Thus,

inflation of the lungs, which has an effect mostly on slowly adapting receptors under normal conditions, is usually associated with bronchodilatation (214), although differential blockade of the slowly adapting receptors (and rapidly adapting receptors) by vagal cooling has been reported to result in bronchoconstriction due to the unopposed effects of the non-myelinated afferents (202).

Deflation stimulates some rapidly adapting receptors and non-myelinated afferents and may be associated with an increased bronchomotor tone (93,199). The effects of pulmonary congestion is reported to be bronchoconstrictive (5,6), the mechanism of which requires further study and forms part of the investigation of this thesis.

3. Effects of Lung Autocoids

Administration of two commonly-used lung autocoids, histamine and bradykinin, has been associated with bronchoconstriction (147,215, 216). The mechanism of action of histamine is generally thought to be due to stimulation of the rapidly adapting receptors (217) but histamine also has a direct effect of the airway smooth muscle by stimulating the H_1 and H_2 receptors thought to be present in airway smooth muscle (218). In dogs, the effect of bradykinin is considered to be due to stimulation of the bronchial C-fibre receptors (147).

4. Effects of Foreign Airway Irritants

A number of inhaled irritants, such as fumes, smoke and dust, are thought to evoke bronchoconstriction by activation of the pulmonary rapidly adapting receptors (217).

5. Effects of Peripheral Chemoreceptors, Baroreceptors and Other Afferent Inputs

Hypercapnoea has been reported to have a direct bronchodilatating effect which may be masked by (i) the reflex bronchoconstricting effect of carbon dioxide and (ii) the often accompanying hypoxia which has a bronchoconstricting effect (219). The effects of carbon dioxide appear to depend on the concentration of the carbon dioxide inhaled as well as the possible site of action of carbon dioxide, i.e., dependent upon the experimental conditions (220).

A drop in pH, i.e., an increase in $[H]^+$ has been shown to result in dilatation of isolated airways while an increase in pH has the opposite effect (221)

Stimulation of the carotid-sinus baroreceptors has been shown to cause a small dilatation of the trachea (222). Recently, it has been reported in dogs that, decreasing the mean carotid-sinus pressure below a control level of 100-110 mm Hg produced increases in tracheal tension whereas increasing the carotid-sinus pressure above the control level had the opposite effect in which the aortic nerves had been interrupted (223).

Another study suggested that infusion of hypertonic saline solution into the pulmonary artery could cause a reflex bronchoconstriction due to stimulation of the RAR (224).

6. Effects of Splanchnic and Peripheral Chemosensitive Afferents

Direct application of capsaicin and bradykinin to the serosal surfaces of the abdominal organs have been shown to result in reflex relaxation of tracheal smooth muscle (225). Muscular contraction of the limbs has also been shown to relax tracheal smooth muscle

reflexly in dogs due to stimulation of the non-myelinated afferents in the muscles (226).

D. Effects of Administered Chemicals

Besides the effects of atropine, histamine and bradykinin on tracheobronchomotor tone that have been mentioned, a number of chemicals, administered intravenously in the intact animal or studied on preparations of isolated airway muscle in a tissue bath, have effects on the smooth muscle tone. Isoprenaline (isoproterenol) causes the relaxation of the airway smooth muscle as a result of stimulation of the beta$_2$-adrenergic receptors (227). Blockade of adrenergic agonists such as isoprenaline, noradrenaline (norepinephrine) and phenylephrine by alpha and beta blocking agents, showed that, with selective alpha blockade, relaxation of tracheal smooth muscle that is selectively reversed by beta blockade occurred (197). Alpha-receptor stimulation caused bronchoconstriction in some patients with obstructive airway disease (228). However, another study with propranolol on adrenal demedullated rats suggested that the bronchoconstricting effects of this drug are unrelated to the beta-adrenergic blockade of the airway smooth muscle, a finding that is hard to reconcile with the generally accepted views of the effects of beta-blocking agents in causing bronchoconstriction (229). This suggests that more than one mechanism may be operative in the action of the adrenergic agonists and antagonists (229).

PGI_2 have been shown to have a bronchodilatating effect when given as an aerosol to human asthmatic subjects (230). However, a paradoxical bronchoconstricting effect was found with PGE_1 and PGI_2 when they were infused intravenously, the mechanism of

action of which has been postulated to be due to stimulation of the bronchial C-fibre receptors (231).

E. Implications in Asthma - Bronchial and Cardiac

These two forms of asthma have superficial similarities in that in both cases, patients present with respiratory wheeze, increased respiratory efforts and may present in acute respiratory distress.

Bronchial asthma is a disease of the airways in which hyper-reactivity of the tracheobronchial smooth muscle plays a role. It is thought that inhaled allergens set up an allergic reaction leading to the release of mediators such as histamine and prostaglandins which in turn activate sensory endings in the epithelium of the airways (84,215,232). The afferents involved are thought to be RAR, pulmonary C-fibre and bronchial C-fibre receptors. The result of stimulation of these receptors is a reflex bronchoconstriction and an increase in airway secretions both of which have an effect in obstructing the airway lumen (217). In humans, the evidence for this mediator-induced reflex bronchoconstriction is that inhalation of many mediators such as prostaglandins, leukotrienes, bradykinin and 5-hydroxytryptamine induces asthmatic attacks in susceptible subjects (217,232). The suggestion that this is a reflex is supported by the finding that atropinic drugs, given in adequate dosages to reach appropriate sites in the airway, can be effective in inhibiting allergen and mediator-induced bronchoconstriction in a proportion of subjects (233).

In cardiac asthma, the initiating event is pulmonary venous congestion leading to pulmonary interstitial oedema. In this

situation, a reduction in the airway lumen is attributed to one or more of the following possible mechanisms :

1. Intraluminal obstruction due to the accumulation of oedema fluid in the interstitial space causing mucosal swelling (2),
2. Extrinsic compression on the airways as a result of the development of peribronchial oedema and vascular engorgement (3),
3. Decrease in radial traction as a consequence of the increased "stiffening of the lungs" from the fluid and blood accumulating in the interstitial spaces and resulting in a reduced functional residual capacity of the lungs (4).

There is reason to believe that a reflex bronchoconstriction may also occur during cardiac asthma (5,6). To determine whether this is indeed the case forms part of the investigation in this study.

Summary on Tracheobronchial Smooth Muscle

(1) The calibre of the airways depends on the smooth muscle tone of the tracheobronchial tree.

(2) A number of methods have been used to monitor this change in muscle tone.

(3) The airway smooth muscle is under the influence of a dual autonomic nervous control. However, the extent of innervation of the smooth muscle into the smaller branches of the airways varies in different animal species.

(4) Tracheobronchial smooth muscle tone is under the influence of a number of sensory inputs, i.e., afferents from the lungs and airways, chemoreceptors, baroreceptors and other peripheral afferents, as well as of lung autocoids and administered "foreign" chemicals.

(5) Evidence is available that, in a proportion of cases of human subjects, bronchial asthma is due to an antigen-antibody reaction releasing mediators inducing a reflex bronchoconstriction. While the mechanism in cardiac asthma is thought to be due to the effect of interstitial oedema on the airway lumen, a reflex bronchoconstriction cannot be ruled-out. The study of this reflex bronchoconstriction forms part of the investigations of this study.

STATEMENT OF THE PROBLEM

The review of the literature has produced unequivocal evidence that the pulmonary receptors respond to various stimuli applied directly to the respiratory system. However, the responses of these receptors to a small degree of acute, sustained pulmonary venous congestion, in the absence of alveolar oedema, in not clear. Further, the presence of reflex response(s) of airway smooth muscle to this sustained pulmonary venous congestion has not been demonstrated directly.

Therefore, the present series of experiments were designed to study:

1. The responses of the pulmonary receptors to an acute sustained pulmonary venous congestion produced by elevating the left atrial pressure by up to 15 mm Hg above control level for a period of 15 min.
2. The responses of the tracheal smooth muscle to the above pulmonary venous congestion.
3. The effects of pulmonary venous congestion on pulmonary lymphatic flow and the dynamic compliance of the lungs.

It is intended to produce pulmonary venous congestion by occluding partially the mitral valve and elevating the left atrial pressure.

CHAPTER 7

GENERAL EXPERIMENTAL METHODS

This section describes the methods used in the series of experiments in this study. Following this chapter dealing with the general experimental methods involving the techniques common to all the experiments, specific techniques particular to each protocol will be described in subsequent chapters. In the chapters following this, each will deal with a specific protocol and will start with a brief introduction outlining the purpose of the protocol. This introduction is followed by a description of techniques specific to that protocol, details of experimental protocols and the appropriate statistical methods used. Finally, the results obtained will be reported.

Premedication and Anaesthesia

Dogs weighing 18-32 kg were premedicated with an injection of morphine sulphate (0.5 mg/kg subcutaneously). Thirty minutes later, they were prepared for general anaesthesia with alpha-chloralose (Fisher Scientific). Under local anaesthesia (lidocaine, dose: 10 mg xylocaine, 2% w/v; Astra Pharmaceutical Division, Mississauga, Ontario), a cannula (ID 1.57 mm, Intramedic polyethylene tubing, Clay Adams, Parsipanny, New Jersey) was inserted into the inferior vena cava via the left saphenous vein.

The alpha-chloralose (5.0 gm) was dissolved in 500 ml of NaCl solution (0.9 w/v, in distilled water) at a temperature between 65 and 70°C. To facilitate the solution of the alpha-chloralose, the mixture was stirred continuously. The solution was then transferred to a distillation column bearing a filter at the lower outflow end.

The column was maintained at 50°C by circulating water through its outer jacket. This procedure ensured that the anaesthetic solution was injected into the animal at a temperature of 45-47°C, as measured at the proximal end of the cannula in the saphenous vein. The initial dose of alpha-chloralose was 0.1 gm/kg. Subsequently, a state of light anaesthesia was maintained by a continuous infusion of alpha-chloralose (dose 0.05-0.075 ml/kg/min). The dogs were not paralysed.

Alpha-chloralose is associated with acidaemia and this was prevented in this study by a slow constant infusion of sodium bicarbonate (7.5% w/v, 0.1 ml/min).

Artificial Ventilation

As soon as possible following the induction of anaesthesia, the dogs were cannulated with a cuffed endotracheal tube (ID 10 mm, length 20 cm; National Catheter, Argyle, New York) and ventilated artificially (constant volume intermittent positive pressure ventilation) using a Harvard ventilator (Model 607, Harvard Instruments, Millis, Massachusetts) at the rate of 18/min and with a tidal volume of 12-15 ml/kg. The open end of the expiratory tube of the ventilator was placed under 2 cm of water in order to prevent collapse of the lungs in the dog with the open chest. Supplemental oxygen was given to maintain an adequate level of arterial pO_2. A cannula (ID 1.67 mm, Intramedic polyethylene tubing) was positioned in the trachea so that this open end was at the level of the carina while the other end was connected to a pressure transducer for the measurement of intra-tracheal airway pressure (Fig.1).

The dogs were ventilated by a constant volume ventilator. It was

found that, under the atmospheric conditons prevalent here in Edmonton, a tidal volume of 12 to 15 ml and a ventilation rate of 18 cycles/min were adequate to ventilate the dog via an endotracheal tube. When the dog was ventilated via a tracheostomy tube, the tidal volume could be reduced, as was done in Protocol IV.

Arterial blood gas measurements were made at the start of each experiment and then at periodic intervals (Instrumentation Laboratory Inc., pH/Blood Gas Analyzer 813, Lexington, Massachusetts). Once the blood gases of the animals were adjusted to the normal range, the ventilatory parameters were not changed, so as not to distort the experimental recordings.

Measurement of Cardiovascular Parameters

A cannula (ID 1.67 mm, Intramedic polyethylene tubing) was inserted into the right femoral artery and advanced into the aorta. This cannula was used for recording the aortic pressure. Cannulae were each inserted into the right femoral vein and advanced into the inferior vena cava, and into the right external jugular vein to the right atrium. These cannulae were used for injection of drugs, sodium bicarbonate and intravenous fluid.

The chest was opened in the midline and a latex balloon attached to the end of a polyethylene cannula (ID 1.67 mm, Intramedic polyethylene tubing) was inserted into the left atrium through the left atrial appendage. A second cannula (ID 1.67 mm, Intramedic polyethylene tubing) was inserted into the left atrium and used for recording the pressure in the left atrium.

The cannulae for recording pressure were connected to transducers

(Model P23 DB, Statham Instruments Ltd., Hato Rey, Puerto Rico) the output of which was amplified and recorded on light sensitive paper (Model VR12, Electronics for Medicine/Honeywell, Pleasantville, New York). The frequency response of the system for recording pressure was flat to 50Hz (±2%). Both "real time" pressures and mean pressure for intra-tracheal, aortic and left atrial pressures could be monitored and recorded. Mean pressures were calculated electronically. The zero values for the atrial pressure was obtained post-mortem after exposure of the tip of the cannula to the atmosphere.

While mean aortic (arterial), atrial and intra-tracheal pressures were calculated electronically, the actual measurements were recorded and, where necessary, measured manually to avoid errors due to the "averaging" effect of the monitoring system. This was particularly important with the low pressure readings such as intra-airway and atrial pressures.

Heart Rate

The heart rate was continuously monitored from an electrocardiogram (standard lead II) using a cardiotachometer in the same system (Model VR12, Electronics for Medicine/Honeywell) and recordings were made.

Temperature Control

The temperature of the animal was monitored using a thermistor placed in the oesophagus (Model 43TD, Yellow Springs Instruments Co., Yellow Springs, Ohio). The temperature of the animal was maintained at 37 ± 1°C using heating lamps.

Production of Pulmonary Venous Congestion

The cannulae in the left atrium, one bearing a balloon at its end and the other attached to a pressure transducer for measurement of left atrial pressure were anchored firmly by a clamp to prevent displacement of the inflated balloon into the mitral valve apparatus and disruption of the circulation. Pressure in the left atrium was increased by gradually distending the balloon with 10 to 20 ml of 0.9% NaCl solution. This manoeuvre produced partial obstruction of the mitral valve. Elevation of the left atrial pressure resulted in increased pressure in the pulmonary veins and caused pulmonary venous congestion (Fig. 2).

CHAPTER 8

Protocol 1. The Activity of the Pulmonary Receptors During Pulmonary Venous Congestion

The series of experiments in this section investigated the effects of pulmonary venous congestion on the four types of pulmonary receptors, the effects of graded increases in left atrial pressure.

After the dog had been prepared as described in the chapter on General Experimental Methods, action potentials were recorded from branches of the cervical vagus as follows:

Recording Action Potentials

The vagus nerve (left or right) was exposed in the neck via a ventral midline incision. The vagus nerve was dissected away from the common carotid artery. A pool of paraffin (38°C) was made from the skin flap and surrounding tissue to cover the nerve and prevent it from drying. The vagus nerve was placed on a black perspex dissecting platform which was placed within the pool (Fig.1). The rest of the procedure was carried out using a dissecting microscope.

The blood vessels on the surface of the nerve were ligated on either side of the platform and the sheath of the nerve was carefully dissected away for a length of 1-1.5 cm from the nerve until the bundles of nerve fibres were seen as shiny strands. Slips of the vagus were prepared in the following way. A nerve bundle was cut from the nerve trunk at a point cranial to the platform and after being separated caudally from the main trunk, was laid on the perspex platform. The cut end of the bundle was gently teased apart and grasped with two pairs of fine forceps. The bundle was then split by separating the forceps. This subdivision was repeated several times

and each fine slip was placed on a pair of bipolar silver electrodes to determine its impulse activity. This fine dissection of the nerve bundle was continued until single units were obtained.

Action potentials were recorded using previously established techniques (156,234). Slips of the cervical vagus were placed on silver electrodes and the output was amplified (Tektronix Type 122, Low Level Preamplifier, Tektronix Inc., Oregon) and recorded (Electronics for Medicine/Honeywell). The action potentials were also displayed on an oscilloscope in addition to being fed into an audio amplifier (Model 32-2025, Tandy Electronics, Barrie, Ontario). In order to facilitate the calculation of the activity in the pulmonary receptors, the signals were fed into a discriminator and counted electronically. The fidelity of the recordings was established by recording simultaneously both the "raw" and the "processed' signals.

Determination of Conduction Velocity

The conduction velocity in the fibres was measured by stimulating the vagus electrically (Nerve Stimulator Model SD9B Grass Inst. Co., Quincy, Massachusetts) at a point 4-5 cm caudal to the site of the recording. In order to minimize the current spread during stimulation of the nerve, the vagus was grounded between the recording and stimulating electrodes at a point 1 cm from the latter. The site of stimulation was not de-sheathed. The action potential evoked in the nerve was monitored on an oscilloscope which was triggered by the stimulus. The stimulus parameters were as follows: myelinated fibres, duration 0.05-0.10 msec, strength 8-80 V (17.8 $\pm$ 3.7 V); non-myelinated fibres, duration 0.5-1.0 msec, strength 50-80 V (58.0 $\pm$ 2.2 V). time taken for the impulse to traverse the length

of the nerve from the site of stimulation to the recording site was obtained from the oscilloscope. The distance between the site of recording and the point of stimulation was also measured. These two values were used to calculate the conduction velocity. The identity of the unit stimulated was established in two ways. In the case of the SAR which had a "constant" rate of discharge, it was possible to demonstrate "collision". For the other types of fibres examined, the identification was based upon amplitude and shape of the action potential discharges (see below). The above procedures permitted the differentiation of myelinated from non-myelinated fibres.

Identification of the Receptors

The SAR and RAR were identified usually from the pattern of afferent activity recorded from the cervical vagus nerve and from their conduction velocities. These units were differentiated further by their responses to stepwise sustained inflation of the lungs in the manner of Knowlton and Larrabee (55): the SAR showed a sustained increase in the pattern of discharge while the RAR exhibited evidence of rapid adaptation. An adaptation index was obtained by hyperinflating the lungs in a stepwise manner by occluding the expiratory line of the ventilator for three ventilatory cycles. The ventilator was switched off at peak inspiration and the inflation maintained for 5s. Experimental recordings were obtained at a paper speed of 50 mm/s and the adaptation index (A.I.) was calculated using the following formula:

$$A.I. = \frac{\text{Peak Frequency - Average Frequency during 2nd second}}{\text{Peak Frequency}} \times 100\%$$

Peak frequency was calculated over 0.2s and coincided with the peak of inflation. The 2nd second was defined as the period between 1.2s and 2.2s following the start of the measurement.

The non-myelinated C-fibres had conduction velocities less than 2 m/s and their pattern of discharge in relation to the ventilatory rhythm was random. Pulmonary C-fibre receptors were identified further by their rapid response (less than 3 seconds) following injection of capsaicin (10 ug/kg; capsaicine natural, Fluka AG, Chemische Fabrik, Switzerland) into the right atrium (60,134,146). Bronchial C-fibre receptors were identified by their response within 7-12 seconds to an injection of phenyldiguanide (10 ug/kg; 1-phenyldiguanide hydrochloride, Fluka AG, Chemische Fabrik, Switzerland) into the right atrium (65,157). Phenyldiguanide solution was prepared by dissolving 10 mg phenyldiguanide hydrochloride in 100 ml 0.9% NaCl solution. Capsaicin was dissolved in NaCl solution (10 mg capsaicin in 98 ml 0.9% saline solution and 2 ml absolute ethanol) kept at 65 to 70°C.

Experimental Protocols

Three protocols were carried out in this series of experiments. In all cases, after an afferent unit having one of the above characteristics was identified, the preparation was left for 10 minutes for equilibration. Next, one of the following protocols was completed.

Protocol 1A. Activity of Pulmonary Receptors during Acute Elevation of Left Atrial Pressure for 15 minutes

Observations were made of the responses of all four types of pulmonary receptors to an elevation of left atrial pressure and the resultant pulmonary congestion. Recordings were made for an initial control period of 10 minutes. At the end of this period, the balloon in the left atrium was distended to increase the left atrial pressure by approximately 10 mm Hg. After 1 min, recordings were made for a period of 15 min. At the end of this period, the balloon was deflated and after a period of 1 min, recordings were made for a second control period of 10 min.

Minute by minute recordings of the nerve action potentials, peak and mean intra-tracheal pressure, mean left atrial pressure, mean aortic pressures and heart rate were made for the duration of the protocol.

Protocol 1B. Effects of Graded Increases in Left Atrial Pressure on Pulmonary Receptor Activity

In this protocol, the effect of a graded increment of left atrial pressure on the discharge of the four types of pulmonary receptors was examined. After obtaining records for an initial control period of 5 min, the balloon in the left atrium was distended to increase the left atrial pressure in steps of 5 mm Hg (i.e. 5, 10 and 15 mm Hg) up to a maximum increase of 15 mm Hg. Each step was maintained for 5 min.

Minute by minute recordings of nerve action potentials, peak and mean intra-tracheal pressure, mean left atrial pressure, mean aortic pressure and heart rate were made as in Protocol 1A above.

Protocol 1C. Effects of Vagal Cooling, Vagotomy and Bilateral Carotid Clamping on RAR Activity

This protocol was designed after analysis of the results of Protocol 1A showed that the RAR had the greatest increase in activity in response to the increments in the left atrial pressure. The following additional procedures were carried out to establish the possible mechanisms involved in this phenomenon.

(a) After demonstrating the response to an increase in left atrial presssure of 10 mm Hg (5 min) as in Protocol 1A, the procedure was repeated after cooling both vagi to 8°C. The vagi were rewarmed and the stimulus was applied once more. The vagi were then sectioned and the procedure repeated. On the nerve from which recordings were made, both procedures were done cranial to the site.

Vagal Cooling: The vagus nerves were freed for 3-4 cm from the cervical carotid sheaths. Each nerve was then placed in the groove of a cooling platform (width 2.5 cm) which was cooled by a thermode consisting of a thermocouple arrangement. The temperature of each platform was measured by a small thermistor (Model YSI 511, Yellow Springs Instruments Co. Inc., Yellow Springs, Ohio) which was placed inside the groove in contact with the nerve.

The method of vagal cooling used in this study is well established (156). The nerve was cooled in the groove of a silver cooling platform attached to a thermocouple arrangement. When electric current passes through this apparatus, cooling of the thermode occurred and the excess heat was removed by a constant stream of cold water flowing adjacent to the apparatus. The rate of cooling was controlled by adjusting the strength of current passing

through. The temperature of the nerve was monitored by a small thermistor lying in a groove adjacent to the nerve. The temperature scale of the thermistor was calibrated against a standard laboratory mercury thermometer at two points: 10°C and 0°C before each session. It was found that the temperature of the nerve could be monitored accurately in this way (156).

(b) The activity in the RAR was recorded over a 2 min control period. The common carotid arteries were occluded by clamps for 30s and the effect of this manoeuvre on the activity of the RAR was noted. The clamps were then released and further control recordings made for 5 min.

Clamping Carotid Arteries: Both common carotid arteries were dissected out in the neck and separated from the vagi. Two spring loaded clamps (Crile Hemostat, size 5.5 inches, Lawton GmbH & Co., Tuttlingen, Germany) were positioned on the arteries. Both clamps were applied simultaneously without additional tension on the length of the arteries. When they were removed, care was taken to synchronize the removal on both sides. The time of application and removal were defined on the recording by an event marker.

Location of Receptors in the Lung

After completion of the above protocols, the location of the receptors was established by discrete palpation of the mediastinum and the lungs. In the case of the SAR and the RAR the localisation was limited to a segment of the bronchus approximately one cm in length. In the case of the pulmonary C-fibre and bronchial C-fibre receptors, the process was continued until the receptor was localised to a segment of parenchymal tissue of the lung.

STATISTICAL ANALYSIS

Group data was expressed as means $\pm$ standard errors of the means.

In all cases, a p value < 0.05 was accepted as indicative of statistical significance. In protocols IA, IB and IC, where two or more treatments were compared by an analysis of variance (ANOVA), the Least Significant Difference test (LSD) at $p < 0.05$ was used to detect differences between means, if the ANOVA was significant (235).

The activity in the nerve fibres was expressed in terms of the number of action potentials/ventilatory cycle (also "action potentials/s"). The settings of the ventilator were unchanged (18 ventilatory cycles/minute) after recording commenced. In all protocols, the data obtained during the experimental periods was compared to those obtained during the control periods.

In Protocol IA, a further analysis was done after dividing the receptor activity into the inspiratory and expiratory phases of ventilation. This separation was done on the basis of the changes in intra-tracheal pressure. A factorial analysis was undertaken to establish the effects of the two phases of ventilation and elevation upon receptor activity. This approach was used to analyse separately the effects of pulmonary congestion on receptor activity during the two phases of ventilation.

Heart rate, mean aortic pressure, mean left atrial pressure, mean and peak intra-tracheal pressures were compared using an analysis of variance (see above). Arterial blood gases parameters were analysed by a paired t-test comparing the data obtained from the initial and treatment periods.

RESULTS

Protocol IA. Activity in Pulmonary Receptors during Acute Elevation of Left Atrial Pressure for 15 minutes

Protocol IA was completed in 32 dogs. At the start of the recordings, heart rate, mean arterial pressure and left atrial pressure were 150±3 beat/min, 111±3 mm Hg and 7.5±0.2 mm Hg respectively. Arterial blood pH, pCO_2 and pO_2 were 7.33±0.01, 34.7±1.4 mm Hg and 212.6±11.9 mm Hg respectively. The average increase in left atrial pressure was 9.4±0.2 mm Hg.

Activity in SAR

Fifteen SAR were examined. Activity in the units was related to expansion of the lung and in all these units, activity during inspiration was greater than during expiration. All units showed sustained activity for a maintained inflation and had adaptation indices <20% (Fig.3A). Average conduction velocity in these fibres was 22.2±4.2 m/s (range 7.5-45.8 m/s).

During the control period, average activity was 69.3±8.5 action potentials/ventilatory cycle (20.8±2.6 action potentials/s); inspiratory phase, 46.6±5.4 action potentials/cycle and expiratory phase, 22.5±3.7 action potential/cycle. When left atrial pressure was elevated, 14 of the 15 receptors examined showed increases in activity. Average activity increased to 79.3±8.6 action potentials/cycle (23.8±2.6 action potentials/s); inspiratory phase, 49.2±5.6 action potentials/ cycle and expiratory phase, 30.2±4.1 action potentials/cycle. The increases in activity per ventilatory cycle was statistically significant ($p<0.005$) as was the increase during the expiratory phase ($p<0.01$). The change during the

inspiratory phase was not significant. When left atrial pressure was restored to control, the activity returned to values which were not significantly different from the initial control values [i.e. 68.2±8.0 action potentials/cycle (20.5±2.4 action potentials/s); inspiratory phase, 44.5±5.5 action potentials/cyle and expiratory phase, 23.9±3.2 action potentials/cycle]. An example of one such unit is shown in Fig. 5A. The results of all the SAR are summarized in Fig. 9 and 13.

When left atrial pressure was elevated, activities during both phases of ventilation increased, but that during the expiratory phase showed a greater proportional increase. In two SAR, the activity during the expiratory phase became greater than that during inspiration, due to a change in discharge pattern from phasic to sustained activity. During the final control period, activity decreased during both phases of ventilation (as compared with the period of elevated left atrial pressure) but one of the two SAR which showed sustained activity during congestion continued to be more active during expiration than during inspiration.

Activity in RAR

Eleven RAR were examined under the same conditions as the SAR. The activity in these units had a respiratory rhythm but this relationship was less consistent than in the SAR. Of the 11 RAR studied, 9 showed predominantly inspiratory activity and 2 predominantly expiratory activity during the initial control period. All units showed evidence of rapid adaptation (Fig. 3B) and the adaptation index in these RAR was 82.6±2.6% (range 73-91%). Average conduction velocity in these fibres was 25.6±10.4 m/s (range

13.6-40.0 m/s).

During the initial control period, average activity was 12.3±4.2 action potentials/cycle (3.7±1.2 action potentials/s); inspiratory phase, 8.8±3.0 action potentials/cycle and expiratory phase, 3.5±1.3 action potentials/cycle. When left atrial pressure was elevated, activity increased to 22.7±5.7 action potentials/cycle (6.8±1.7 action potentials/s); inspiratory phase, 14.5±4.5 action potentials/cycle and expiratory phase 8.2±2.0 action potentials/cycle. These increases are statistically significant ($p<0.005$ for overall changes, $p<0.01$ for change during the inspiratory phase and $p<0.05$ for change during the expiratory phase). After left atrial pressure was restored to control level, activity returned to values which were not different from those during the initial control period [i.e., 11.5±3.5 action potentials/cycle (3.4±1.0 action potentials/s); inspiratory phase, 5.9±2.2 action potentials/cycle and expiratory phase 5.3±2.0 action potentials/cycle]. An example of a RAR is shown in Fig. 5B. The results of all the RAR are summarized in Fig. 10 and 14.

During the period of elevated left atrial pressure, predominantly inspiratory activity was shown by 4 RAR, predominantly expiratory activity by 4 and equal activity during the two ventilatory phases by 3. During the final control period, 7 RAR showed predominantly inspiratory activity, 3 predominantly expiratory activity and one equal activity during both phases of respiration.

Activity in Bronchial C-fibre Receptors

Nine bronchial C-fibre receptors having an average conduction velocity 1.3±0.1 m/s (range 0.5-1.7 m/s) were examined. All units

were activated 8.8±0.8 seconds after injection of phenyldiguanide into the right atrium (Fig. 4A). Activity in the C-fibre receptors was irregular with no apparent modulation caused by inflation and deflation of the lungs. Nevertheless, during the initial control period, their average activity was 8.2±1.6 action potentials/ventilatory cycle (2.5±0.5 action potentials/s); inspiratory phase, 3.2 ± 0.6 action potentials/cycle and expiratory phase 5.0 ± 1.2 action potentials/ cycle. When left atrial pressure was increased, activity became 11.4 ± 2.0 action potentials/cycle (3.4±0.6 action potentials/s); inspiratory phase, 4.4 ± 0.9 action potentials/cycle and expiratory phase, 6.7 ± 0.9 action potentials/cycle) These increases were statistically significant ($p<0.005$ for overall change, $p<0.01$ for both phases of ventilation). When left atrial pressure was restored to control values, activity in these receptors was similar to that during the initial control period [i.e., 7.4±1.5 action potentials/cycle (2.2±0.4 action potentials/s); inspiratory phase 2.9±0.8 action potentials/cycle and expiratory phase 4.6±1.0 action potentials/ cycle]. An example of a response in a bronchial C-fibre is shown in Fig. 6A. The results of all the bronchial C-fibre receptors are summarized in Fig. 11 and 15.

During the initial control period, 3 bronchial C-fibre receptors showed predominantly inspiratory activity and 6 predominantly expiratory activity. When left atrial pressure was elevated, the proportion of receptors showing predominant activity in either phases of the ventilatory cycle remained unchanged. However, one receptor which showed predominantly inspiratory activity during control now

showed predominantly expiratory activity and another receptor showed the opposite changes. During the final control period, two bronchial C-fibre receptors showed predominantly inspiratory activity and 7 predominantly expiratory activity.

Activity in Pulmonary C-fibre Receptors

Nine pulmonary C-fibre receptors having an average conduction velocity of 1.0 ± 0.05 m/s (range 0.8-1.2 m/s) were examined. They responded to capsaicin 1.8 ± 0.2 seconds after injection of the drug into the right atrium (Fig. 4B). During the control period, these receptors were without any respiratory modulation.

During the initial control period, average activity was 4.6 ± 1.8 action potentials/ventilatory cycle (1.4±0.5 action potentials/s); inspiratory phase, 2.0 ± 0.9 action potentials/cycle and expiratory phase, 2.6 ± 0.9 action potentials/cycle). When left atrial pressure was raised activity was 4.8 ± 1.6 action potentials/cycle (1.4±0.5 action potentials/s). Inspiratory and expiratory phases activities were 2.0 ± 0.8 and 2.8 ± 0.9 action potentials/cycle respectively. These changes were not statistically significant. During the final control period, activity was 4.5±1.8 action potentials/cycle (1,4±1.8 action potentials/s); inspiratory phase, 2.1±1.0 action potentials/cycle and expiratory phase 2.4±0.8 action potentials/cycle. An example of a response in a pulmonary C-fibre is shown in Fig. 6B. the results of all the pulmonary C-fibre receptors are summarized in Fig. 12 and 16.

Two pulmonary C-fibre receptors showed predominantly inspiratory activity, four predominantly expiratory activity and three equal activity in both phases of ventilation during the initial control

period. When left atrial pressure was increased, one receptor showed predominantly expiratory activity and eight predominantly inspiratory activity. During the final control period, two showed predominantly inspiratory activity, six predominantly expiratory activity and one equal activity.

Cardiovascular and Respiratory Parameters during Pulmonary Congestion produced by Partial Obstruction of the Mitral Valve

In general, partial obstruction of the mitral valve was associated with a reduction in arterial pressure (from 110 $\pm$ 4 mm Hg to 97 $\pm$ 4 mm Hg) and an increase in heart rate (from 147 $\pm$ 4 beat/min to 157 $\pm$ 6 beat/min). During final control they were 111 $\pm$ 5 mm Hg and 150 $\pm$ 7 beat/min respectively. These changes from control were not statistically significant ($p>0.05$). Mean left atrial pressure increased significantly from 7.4 $\pm$ 0.2 mm Hg to 16.9 $\pm$ 0.3 mm Hg ($p<0.001$) and during final control, it decreased significantly to 7.6 $\pm$ 0.3 mm Hg ($p<0.001$). In additon, there were small but significant increases in mean and peak intra-tracheal airway pressure. Mean intra-tracheal pressure increased from 2.38 $\pm$ 0.19 mm Hg to 2.60 $\pm$ 0.22 mm Hg ($p<0.01$) and returned to 2.45 $\pm$ 0.21 mm Hg during final control ($p<0.01$). Peak intra-tracheal pressure increased from 5.09 $\pm$ 0.25 mm Hg to 5.66 $\pm$ 0.22 mm Hg ($p<0.001$) and during final control it was 5.13 $\pm$ 0.20 mm Hg.

The results of the arterial blood gases measured during pulmonary congestion did not differ significantly from those measured during control periods. The pH, pCO_2, pO_2, and $[HCO_3^-]$ during control period were 7.33$\pm$0.01, 34.7$\pm$1.4 mm Hg, 212.6$\pm$11.9 mm Hg and 17.2$\pm$ 0.7mmol/L respectively. The corresponding values during

pulmonary congestion were 7.33 ± 0.01, 34.7 ± 0.7 mm Hg, 197.3 ± 14.3 mm Hg and 17.5 ± 0.2 mmol/L (see Table 1).

Protocol 1B. Effects of Graded Increases in Left Atrial Pressure on Pulmonary Receptor Activity

In this protocol, changes in activity of the pulmonary receptors brought about by graded increases in left atrial pressure were examined in 17 dogs. Observations were made for periods of 5 min each during initial control, when left atrial pressure were increased by 5, 10 and 15 mm Hg above control levels and when left atrial pressure was restored back to control (final control).

Seven SAR were studied. The initial and final control activities were 34.0 ± 11.2 action potentials/cycle (10.2±3.4 action potential/s) and 34.5 ± 11.8 action potentials/cycle (10.4±3.5 action potentials/s) respectively. When left atrial pressure was increased by 5 mm Hg, SAR activity was 35.9 ± 12.1 action potentials/cycle (10.8±3.6 action potentials/cycle). At 10 mm Hg above control, it was 38.8 ± 13.6 action potentials/cycle (11.6± 4.1 action potentials/s) and at 15 mm Hg it was 41.8 ± 13.7 actions potentials/cycle (12.5±4.1 action potentials/s). These last 2 values differed significantly from the other 3 observations and also from each other ($p<0.05$) (Table 2 & Fig.7).

Five RAR were examined under this protocol. The initial and final control RAR activities were 7.9 ± 2.9 and 7.1 ± 1.7 action potentials/cycle (2.4±0.9 and 2.1±0.5 action potentials/s) respectively. The activity at a left atrial pressure of 5 mm Hg above control was 12.3 ± 5.4 action potentials/cycle (3.7±1.6 action

potentials/s). This was significantly greater than the final control activity ($p<0.05$) but not significantly greater than that during initial control ($p>0.05$). The activities at left atrial pressures of 10 mm Hg and 15 mm Hg above control were respectively 16.3 $\pm$ 7.4 and 20.4 $\pm$ 8.8 action potentials/cycle (4.9$\pm$2.2 and 6.1$\pm$2.6 action potentials/s), respectively. These last two values were significantly higher than the other 3 ($p<0.05$) but did not differ significantly from each other (Table 2 & Fig.8).

Five bronchial C-fibre receptors were studied. Their initial and final control activities were 8.9 $\pm$ 1.9 and 9.8 $\pm$ 2.0 action potentials/cycle (2.7$\pm$0.6 and 2.9$\pm$0.6 action potentials/s), respectively. The activity at a left atrial pressure of 5 mm Hg above control was 11.6 $\pm$ 2.4 action potentials/cycle (3.5$\pm$0.7 action potentials/s) and this was significantly higher than the activities during the control periods ($p<0.05$). The activities at 10 mm Hg and 15 mm Hg were 16.2 $\pm$ 3.0 and 17.2 $\pm$ 3.1 action potentials/cycle (4.9$\pm$0.9 and 5.2$\pm$0.9 action potentials/s), respectively. These were not significantly different from each other ($p>0.05$) but both were significantly greater than control values ($p<0.05$). In addition, the activity at 15 mm Hg were significantly greater than that at 5 mm Hg ($p,0.05$)

Five pulmonary C-fibre receptors were studied. Their initial and final control activities were 8.8 $\pm$ 4.0 and 9.1 $\pm$ 4.3 action potentials/cycle (2.6$\pm$1.2 and 2.7$\pm$1.3 action potentials/s), respectively. Their activities at 5 mm Hg, 10 mm Hg and 15 mm Hg above control left atrial pressure were 9.2 $\pm$ 4.0, 9.3 $\pm$ 4.0 and 9.9 $\pm$ 4.3 action potentials/cycle respectively (2.8$\pm$1.2,

2.8±1.2 and 3.0±1.3 action potentials/s). There was a trend towards a greater activity when left atrial pressure was increased by graded increments but the changes were not statistically significant.

The findings are summarized in Table 2.

Cardiovascular and Respiratory Parameters observed during Graded Increases in Left Atrial Pressure

At control left atrial pressure of 5.8 ± 0.4 mm Hg, control heart rate was 157 ± 6 beat/min and mean arterial pressure was 109 ± 4 mm Hg. When left atrial pressure was increased to 10.8 ± 0.6 mm Hg, heart rate increased significantly to 168 ± 6 beat/min and mean arterial pressure decreased significantly to 105 ± 4 mm Hg (both $p<0.05$). At a left atrial pressure of 15.3 ± 0.5 mm Hg, heart rate further increased significantly to 176 ± 5 beat/min while mean arterial pressure decreased significantly to 100 ± 4 mm Hg (both $p<0.05$). When the left atrial pressure was raised to 20.0 ± 0.5 mm Hg, the heart rate was 179 ± 5 beat/min and mean arterial pressure was 97 ± 4 mm Hg. When left atrial pressure was returned to the final control level of 5.4 ± 0.4 mm Hg, heart rate and mean arterial pressure were 161 ± 6 beat/min and 110 ± 3 mm Hg respectively. Both final control heart rate and mean arterial pressure were not significantly different from the initial control levels.

Mean and peak intra-tracheal airway pressure also showed graded increases in response to the increases in left atrial pressure. At each stepwise increase in left atrial pressure, the peak intra-tracheal airway pressure showed a small but significant increase. The peak intra-tracheal airway pressures were 5.51 ± 0.30

mm Hg at initial control. When left atrial pressure were increased in stages, peak intra-tracheal airway pressures were 5.59 ± 0.30 mm Hg, 5.76 ± 0.32 mm Hg and 5.99 ± 0.33 mm Hg at left atrial pressures of 5, 10 amd 15 mm Hg above control. The final control peak intra-tracheal airway pressure was 5.62 ± 0.30 mm Hg. Peak intra-tracheal pressures at left atrial pressures 10 mm Hg and 15 mm Hg were signifcantly different from each other and from the other three pressures ($p<0.05$). The peak intra-tracheal pressure at left atrial pressure of 5 mm Hg above control was not significantly greater than that of the initial control period ($p>0.05$) but was significantly greater than that of the final control period ($p<0.05$). The corresponding values for the mean intra-tracheal airway pressures were 2.48 ± 0.27 mm Hg, 2.65 ± 0.26 mm Hg, 2.70 ± 0.28 mm Hg, 2.76 ± 0.29 mm Hg and 2.58 ± 0.28 mm Hg. Mean intra-tracheal pressures when left atrial pressures were 5, 10 and 15 mm Hg above control were significantly higher than those during the control periods ($p<0.05$) but not significantly different from each other ($p>0.05$). In general, the stepwise changes in left atrial pressure were reflected more closely by the changes in peak intra-tracheal airway pressure than by the changes in mean intra-tracheal airway pressure.

The observations are summarized in Table 4.

Protocol IC. Effects of Vagal Cooling, Vagotomy and Bilateral Carotid Clamping on RAR Activity

In this protocol, specific attention was paid to the behaviour of the RAR in response to an increase in left atrial pressure.

In four RAR, activity was examined after interrupting transmission in the cervical vagi above the point of recording in two ways, bilateral vagal cooling and bilateral vagotomy.

Cooling the vagi to 8°C, to block transmission in myelinated afferent fibres, did not influence either the control activity or the response to an increase in left atrial pressure (+10 mm Hg).

In these four RAR, initial and final control activities at body temperature (37°C) were 9.1 ± 2.2 and 10.6 ± 1.5 action potentials/cycle (2.7±0.7 and 3.2±0.5 action potentials/s), respectively. When left atrial pressure was elevated by 10 mm Hg, RAR activity was 18.3 ± 1.8 action potentials/cycle (5.5±0.5 action potentials/s).

When the vagi were cooled to 8°C at a point cranial to the point of recording, control RAR activities were 8.7 ± 0.9 and 7.3 ± 0.7 action potentials/cycle (2.6±0.3 and 2.2±0.2 action potentials/s). These were not significantly different from the control activities at 37°C. When left atrial pressure was elevated by 10 mm Hg, activity increased to 20.0 ± 6.0 action potentials/cycle and this did not differ significantly from the corresponding activity at 37°C. When the left atrial pressure was increased by 10 mm Hg, in both cases the RAR activity was significantly greater than the control activity (both, $p<0.01$).

Following the above procedure, the vagi were rewarmed back to 37°C. Bilateral vagotomy was then performed at the site of cooling. Initial and final control activities of these four RAR after vagotomy were 9.4 ± 1.4 and 8.2 ± 1.0 action potentials/cycle (2.8±0.4 and 2.5±0.3 action potentials/s) respectively. These did not differ

significantly from the control activity before vagotomy. When left atrial pressure was raised by 10 mm Hg, the RAR activity, which increased to 19.7 ± 5.2 action potentials/cycle (5.9±1.6 action potentials/s), was significantly greater than the control activities ($p<0.01$). (Table 3)

The effect of bilateral carotid occlusion on the activity of these four RAR was examined. Activities during initial and final control periods were 11.3 ± 1.0 and 11.0 ± 1.2 action potentials/cyle (3.4±0.3 and 3.3±0.4 action potentials/s). When the carotid arteries were occluded, activity remained unchanged at 11.0 ± 0.9 action potentials/cycle (3.3±0.3 action potentials/s).

The results are summarized in Table 3.

Cardiovascular and Respiratory Parameters during Vagal Cooling, Vagotomy and Bilateral Carotid Occlusion

The changes in the cardiovascular and respiratory parameters when the left atrial pressure was elevated were similar to those noted in protocol 1A. In this protocol, some of the observations did not achieve statistical significance though the magnitudes of changes were similar to those noted in the larger series in protocols 1A and 1B.

When compared to the initial control values, the heart rate increased and mean arterial pressure decreased when left atrial pressure was elevated by about 10 mm Hg. During the final control period, both heart rate and blood pressure returned to levels similar to those noted in the initial control period.

Similarly, both peak and mean intra-tracheal airway pressure increased when left atrial pressure was elevated by 10 mm Hg. It was

noted that the magnitudes of increase when the vagi were at 8°C and after vagotomy were smaller than that noted when the vagi were at body temperature. This observation was made in every animal.

The results are summarized in Table 5.

During carotid occlusion, the heart rate increased, but not significantly, when compared to the initial control. On release of the occlusion, the heart rate returned to a level less than the initial control level ($p<0.05$) due to the effects of the sudden occlusion and release of the carotids on the carotid baroreceptors. In contrast to the reduction in mean arterial pressure during elevation of left atrial pressure, the mean arterial pressure increased significantly during carotid occlusion ($p<0.05$). The results are summarized in Table 6.

Localisation of the Pulmonary Receptors

Of the receptors investigated under Protocol I, 18 SAR, 14 RAR, nine pulmonary C-fibre receptors and 10 bronchial C-fibre receptors were located to the lungs and large airways. All the SAR and RAR were found to be in the main bronchi while the pulmonary C-fibre receptors and bronchial C-fibre receptors were in the lung parenchyma.

Of the SAR, six were found in the trachea and carina, six in the right main bronchus, three in the left main bronchus, two in the right upper lobe bronchus and one in the right lower lobe bronchus. Three of the RAR were located in the trachea and carina, two each in the right and left main bronchi, one in the right upper lobe bronchus, five in the right lower lobe bronchus and one in the left lower lobe bronchus. Two each of the bronchial C-fibre receptors were found in the right upper and lower lobes of the lung, one in the left

upper lobe, three in the left middle lobe and two in the left lower lobe. Of the pulmonary C-fibre receptors, five were found in the right upper lobe and one each in the right middle, right lower, left upper and left lower lobes (Table 7).

Localisation was not attempted in the other units to avoid collapsing the lungs mechanically, as it was felt that such disturbances could affect the behaviour of the subsequent receptors studied in the same dog. As a consequence, in 14 out of the 53 dogs, more than one receptor was examined. Of these, more than one type of receptor was examined in only two dogs.

TABLE 1

Cardiovascular Parameters, Airway Pressures and Arterial Blood Gas Measurements during control and stimulation of pulmonary receptors

	Control	Elevated LAP	Control
LAP (mm Hg)	7.4±0.2	16.9±0.3**	7.6±0.3
HR (b/m)	147±4	157±6	150±7
MAP (mm Hg)	110±4	97±4	111±5
Mean ITAP (mm Hg)	2.38±0.19	2.60±0.22*	2.45±0.21
Peak ITAP (mm Hg)	5.09±0.25	5.66±0.22**	5.13±0.20

Arterial Blood Gases:

	Control+	Elevated LAP
pH	7.33±0.01	7.32±0.01
pCO_2 (mm Hg)	4.7±1.4	34.7±0.7
pO_2 (mm Hg)	212.6±11.9	197.3±14.3
$[HCO_3^-]$ (mmol/L)	17.2±0.7	17.5±0.2

*: $p<0.01$, **: $p<0.001$, compared to controls,

+: Average of the control observations during the 10 minutes before and after LAP elevation.

LAP: Left atrial pressure; HR (b/m): Heart Rate (beat/min);

MAP: Mean Arterial pressure; ITAP: Intra-tracheal Airway Pressure.

TABLE 2

Pulmonary Receptors Activity (action potentials/ventilatory cycle) during Periods of Control and Graded Increases in Left Atrial Pressure

	INCREASES IN LEFT ATRIAL PRESSURE				
	Control	5 mm Hg	10 mm Hg	15mm Hg	Control
SAR (n=7)	$^{a}34.0\pm11.2$ (10.2 ± 3.4)	$^{a}35.9\pm12.1$ (10.8 ± 5.6)	$^{b}38.8\pm13.6$ (11.6 ± 4.1)	$^{c}41.8\pm13.7$ (12.5 ± 4.1)	$^{a}34.5\pm11.8$ (10.4 ± 3.5)
RAR (n=5)	$^{ab}7.9\pm2.9$ (2.4 ± 0.9)	$^{b}12.3\pm5.4$ (3.7 ± 1.6)	$^{c}16.3\pm7.4$ (4.9 ± 2.2)	$^{c}20.4\pm8.8$ (6.1 ± 2.6)	$^{a}7.1\pm1.7$ (2.1 ± 0.5)
Bronchial C-Fibre (n=5)	$^{a}8.9\pm1.9$ (2.7 ± 0.6)	$^{b}11.6\pm2.4$ (3.5 ± 0.7)	$^{bc}16.2\pm3.0$ (4.9 ± 0.9)	$^{c}17.2\pm3.1$ (5.2 ± 0.9)	$^{a}9.8\pm2.0$ (2.9 ± 0.6)
Pulmonary C-Fibre (n=5)	$^{a}8.8\pm4.0$ (2.6 ± 1.2)	$^{a}9.2\pm4.0$ (2.8 ± 1.2)	$^{a}9.3\pm4.0$ (2.8 ± 1.2)	$^{a}9.9\pm4.5$ (3.0 ± 1.3)	$^{a}9.1\pm4.3$ (2.7 ± 1.3)

Note: In each category of receptors, values with different prefixes are significantly different from each other ($p<0.05$)
Results in parenthesis indicate receptor activity expressed as action potentials/s.

TABLE 3

Effects of (a) Blocking Transmission in the Cervical Vagi on the Responses of RAR to Increases in Left Atrial Pressure, (b) Bilateral Carotid Artery Occlusion on RAR Activity (action potentials/ventilatory cycle; n=4)

(A) VAGAL BLOCK	37°C	At 8°C	Bilateral Vagotomy (37°C)***
Control	9.1±2.2(2.7±0.7)	8.7±0.9(2.6±0.3)	9.4±1.4(2.8±0.4)
Left Atrial Pressure Elevation	18.3±1.8(5.5±0.5)*	20.0±6.0(6.0±1.8)*	19.7±5.2(5.9±1.6)*
Control	10.6±1.5(3.2±0.5)	7.3±0.7(2.2±0.2)	8.2±1.0(2.5±0.3)

(B) CAROTID OCCLUSION	
Control	11.3±1.0 (3.4±0.3)
Carotid Occlusion**	11.6±1.2 (3.3±0.4)
Control	11.0±0.9 (3.3±0.3)

* Significantly increased from controls ($p<0.01$),

** During carotid occlusion left atrial pressure was not changed,

*** Vagotomy performed on the side of recording above the site,

Results in parenthesis indicate receptor activity expressed as action potentials/s.

TABLE 4

Cardiorespiratory Parameters during Periods of Control and Graded Increases in Left Atrial Pressure

		INCREASES IN LEFT ATRIAL PRESSURE			
	Control	5 mm Hg	10 mm Hg	15mm Hg	Control
LAP (mmHg) (n=5)	$^{a}5.8\pm0.4$	$^{b}10.8\pm0.6$	$^{c}15.3\pm0.5$	$^{d}20.0\pm0.5$	$^{a}5.4\pm0.4$
HR (b/m) (n=7)	$^{a}157\pm6$	$^{b}168\pm6$	$^{c}176\pm5$	$^{c}179\pm5$	$^{a}161\pm6$
MAP (mmHg) (n=5)	$^{a}108\pm4$	$^{b}105\pm4$	$^{c}100\pm4$	$^{c}97\pm4$	$^{a}110\pm3$
Mean ITAP (mmHg)(n=5)	$^{a}2.48\pm0.27$	$^{b}2.65\pm0.25$	$^{b}2.70\pm0.28$	$^{b}2.76\pm0.29$	$^{a}2.58\pm0.28$
Peak ITAP (mmHg)(n=5)	$^{a}5.51\pm0.30$	$^{ab}5.59\pm0.30$	$^{c}5.76\pm0.32$	$^{d}5.99\pm0.33$	$^{a}5.62\pm0.30$

Note: In each parameter, values with different prefixes are significantly different from each other ($p<0.05$)

LAP : Left Atrial Pressure; HR (b/m) : Heart Rate (beat/min);
MAP : Mean Arterial Pressure; ITAP: Intra-tracheal Airway Pressure.

Table 5

Cardiorespiratory Parameters

during Blockade of Transmission in the Cervical Vagi

(during Control and Elevated Left Atrial Pressure)

	Control	Elevated LAP	Control
LAP (mm Hg)	4.0±0.6	15.0±1.3*	4.0±0.5
HR (b/m)	144±12	166±16	146±10
MAP (mm Hg)	90±6	79±9	92±6
Mean ITAP (mm Hg)	2.13±0.39	2.48±0.33	2.18±0.38
Peak ITAP (mm Hg)	5.08±0.46	5.45±0.43	5.12±0.39
VAGAL BLOCKADE			
Cooling to 8°C			
LAP (mm Hg)	4.0±0.5	13.4±0.4*	4.1±0.5
HR (b/m)	139±14	157±12	141±9
MAP (mm Hg)	105±4	81±15	93±9
Mean ITAP (mm Hg)	2.38±0.35	2.48±0.35	2.30±0.39
Peak ITAP (mm Hg)	5.28±0.36	5.50±0.31	5.32±0.26
Vagotomy			
LAP (mm Hg)	4.0±0.6	13.1±1.3*	4.1±0.6
HR (b/m)	149±14	158±16	147±12
MAP (mm Hg)	103±9	82±13	98±9
Mean ITAP (mm Hg)	2.23±0.36	2.40±0.33	2.18±0.36
Peak ITAP (mm Hg)	5.52±0.20	5.75±0.25	5.50±0.26

Note : LAP: Left Atrial Pressure; HR (b/m): Heart Rate (beat/min); MAP: Mean Arterial Pressure; ITAP: Intra-tracheal Airway Pressure

*$P < 0.001$, when compared to controls; no statistically significant differences were noted in the other parameters.

TABLE 6

Cardiorespiratory Parameters during Bilateral Carotid Occlusion

	Control	Carotid Occlusion	Control
LAP (mm Hg)	4.1±0.4	4.1±0.5	4.1±0.4
HR (b/m)	146±11	161±7	122±21*
MAP (mm Hg)	102±4	118±3**	100±4
Mean ITAP (mm Hg)	2.20±0.36	2.28±0.33	2.23±0.39
Peak ITAP (mm Hg)	5.52±0.25	5.53±0.23	5.55±0.25

Note : LAP: Left Atrial Pressure; HR (b/m): Heart Rate (beat/min); MAP: Mean Arterial Pressure; ITAP: Intra-tracheal Airway Pressure

*P < 0.05 when compare to initial control and carotid occlusion;

**P < 0.05 when compared to controls.

TABLE 7

Localisation of Pulmonary Receptors Studied

LOCATION		SAR	RAR	Bronchial C-Fibre	Pulmonary C-Fibre
Trachea-Carina		6	3	-	-
Bronchi:	right main	6	2	-	-
	left main	3	2	-	-
	right upper lobe	2	1	-	-
	right lower lobe	1	5	-	-
	left lower lobe	-	1	-	-
Lungs:	right upper lobe	-	-	2	5
	right middle lobe	-	-	2	1
	right lower lobe	-	-	-	1
	left upper lobe	-	-	1	1
	left middle lobe	-	-	3	-
	left lower lobe	-	-	2	1
TOTAL		18	14	10	9

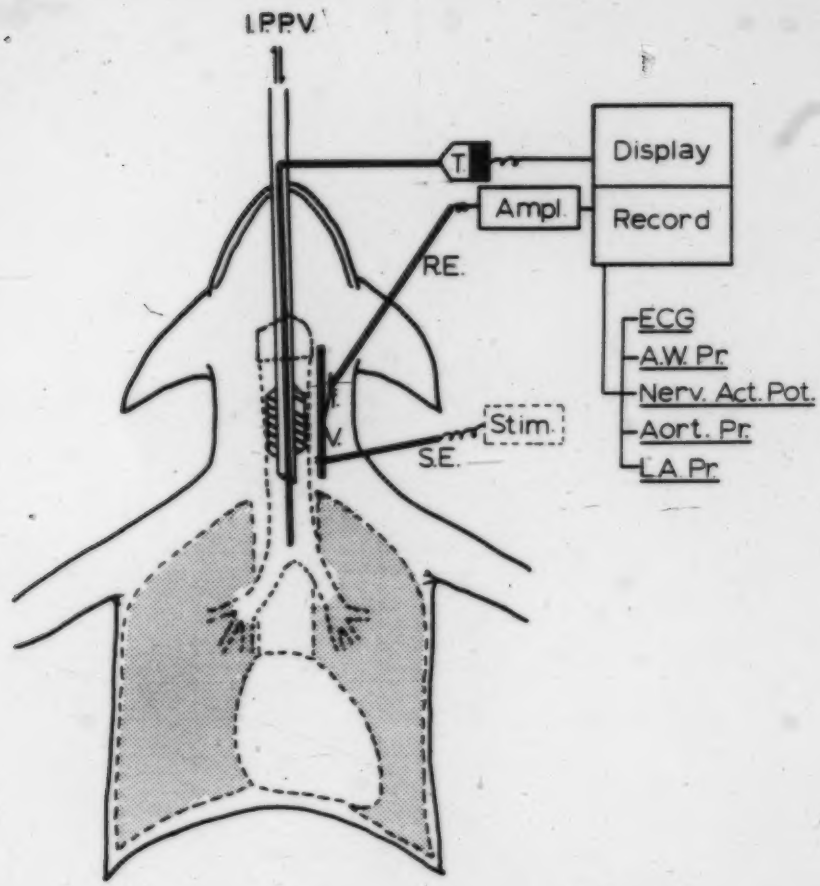

Fig. 1. Experimental preparation for recording nerve activity. The animal was ventilated by intermittent positive pressure ventilation (IPPV) via an endotracheal tube, the cuff of which was inflated to form an air-tight seal. Intra-tracheal airway pressure was monitored as shown by transducer T connected to a cannula with the other end open in the trachea at the carina. The vagus nerve was exposed in the neck via a midline ventral incision. A pool containing paraffin was made and the vagus nerve (V) was desheathed on a dissecting platform under a microscope. A fine nerve bundle (f) was placed on a bipolar electrode (R.E.). A stimulator (Stim.) to stimulate the nerve during measurement of conduction velocity was placed in contact with the vagus nerve caudal to the recording site. The nerve action potentials (Nerve Act. Pot.) were amplified (Ampl.) and together with the ECG, airway pressure (A.W.Pr.), aortic pressure (Aort.Pr.) and left atrial pressure (L.A.Pr.) were displayed on a multichannel oscilloscope and recorded on light sensitive paper.

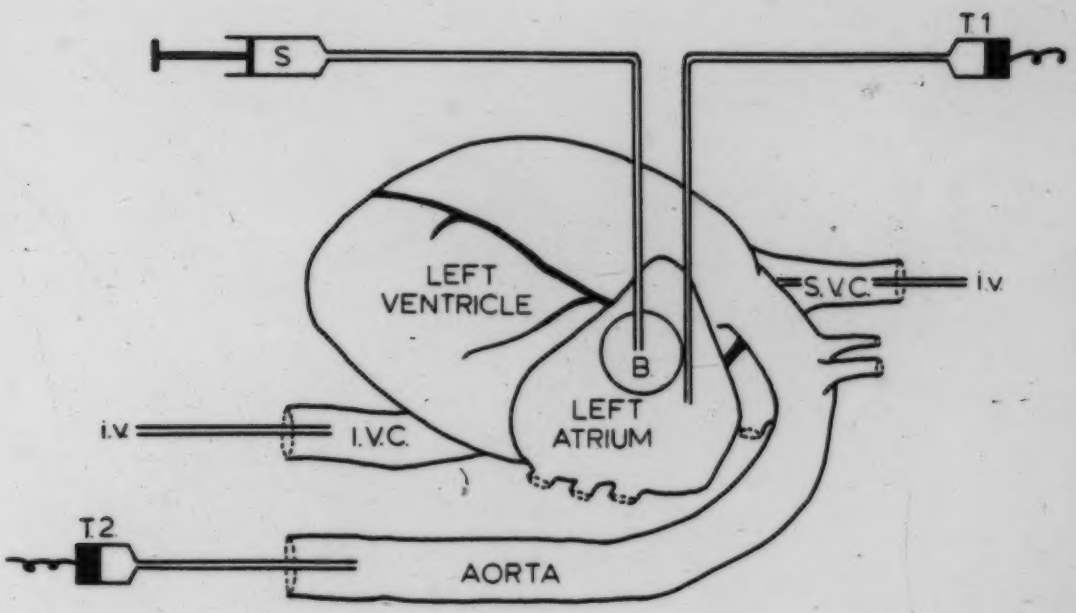

Fig. 2. Partial obstruction of the mitral valve. The chest was opened by a midline sternotomy. A balloon tipped catheter (B) and a pressure transducer cannula (T1) were positioned in the left atrium. An aortic pressure cannula (T2) was inserted via the right femoral artery into the abdominal aorta. Intravenous (i.v.) cannulae were inserted into the inferior vena cava (IVC) and the right atrium via the superior vena cava (SVC) for the administration of fluids and drugs. When balloon (B) was distended, it caused partial obstruction of the mitral valve and elevation of left atrial pressure.

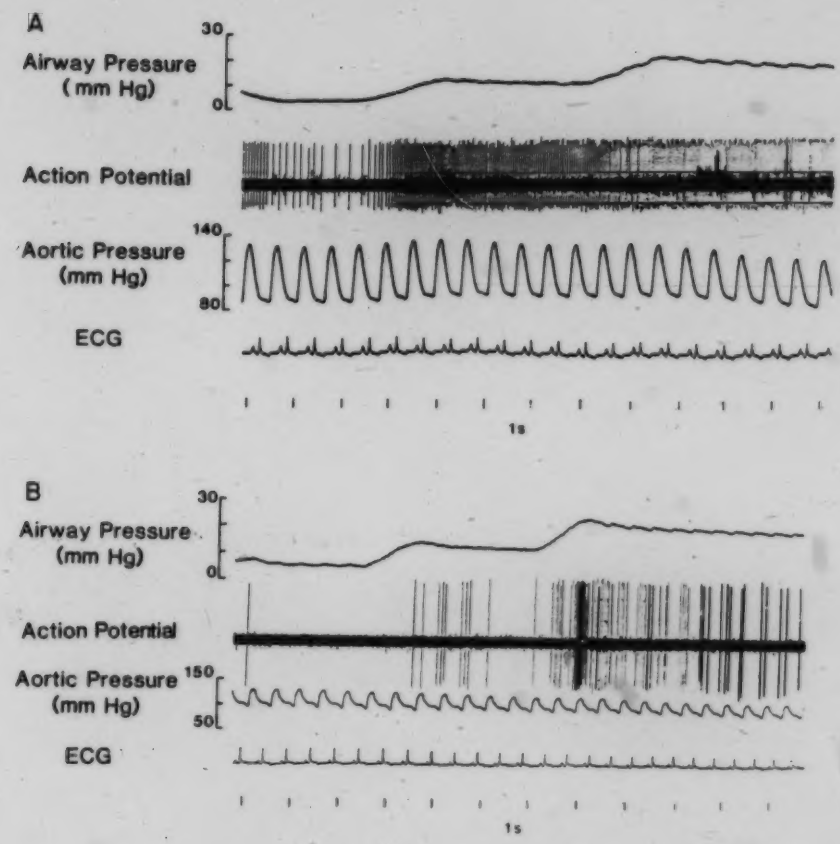

Fig. 3. Examples of SAR (A) and RAR (B) showing the effects of sustained inflation of the lungs. The adaptation index was calculated after 3 breaths (see text). The SAR (conduction velocity 24.3 m/s) showed sustained activity and the RAR (conduction velocity 20.0 m/s) showed adaptation. Each diagram shows, from above downwards : airway pressure (mm Hg), nerve action potentials, aortic pressure (mm Hg), electrocardiogram (ECG) and time (s).

Note: the expiratory line of the ventilator was clamped during the expiratory phase of the first cycle for the SAR in (A) above.

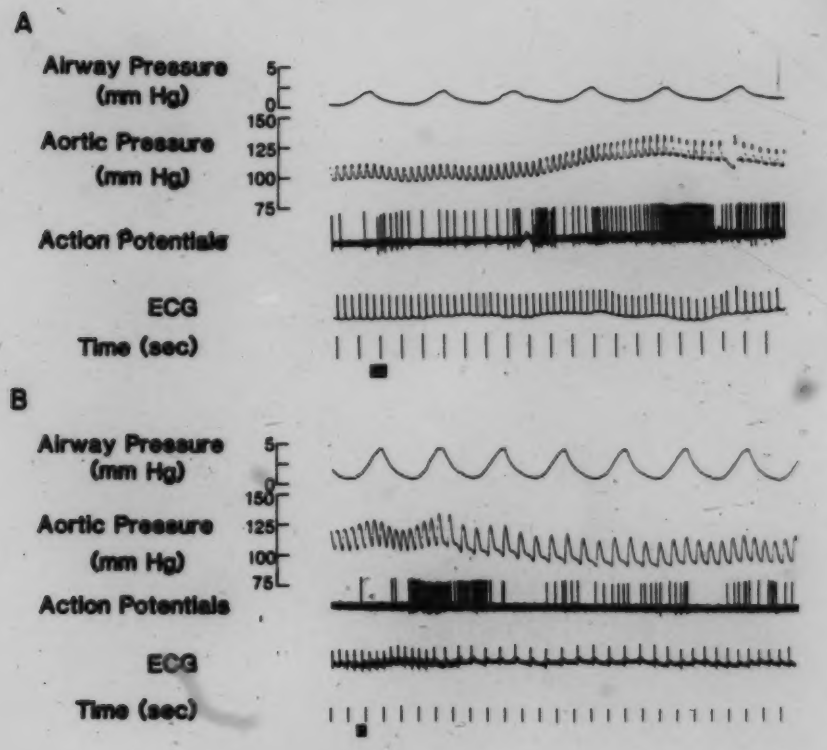

Fig. 4. Examples of a bronchial C-fibre receptor (A) and a pulmonary C-fibre receptor (B), showing the effects of administration of phenyldiguanide (A) and capsaicin (B) into the right atrium. The bronchial C-fibre receptor (conduction velocity 1.7 m/s) showed increased activity 7.5 s after phenyldiguanide and the pulmonary C-fibre receptor (conduction velocity 1.1 m/s) showed increased activity 2.0 s after capsaicin. Each diagram shows, from above downwards : airway pressure (mm Hg), aortic pressure (mm Hg), nerve action potentials, electrocardiogram (ECG), time (s) and marker indicating administration of drugs

Note: In order to enhance clarity during reproduction, some parts of the action potentials have been re-touched.

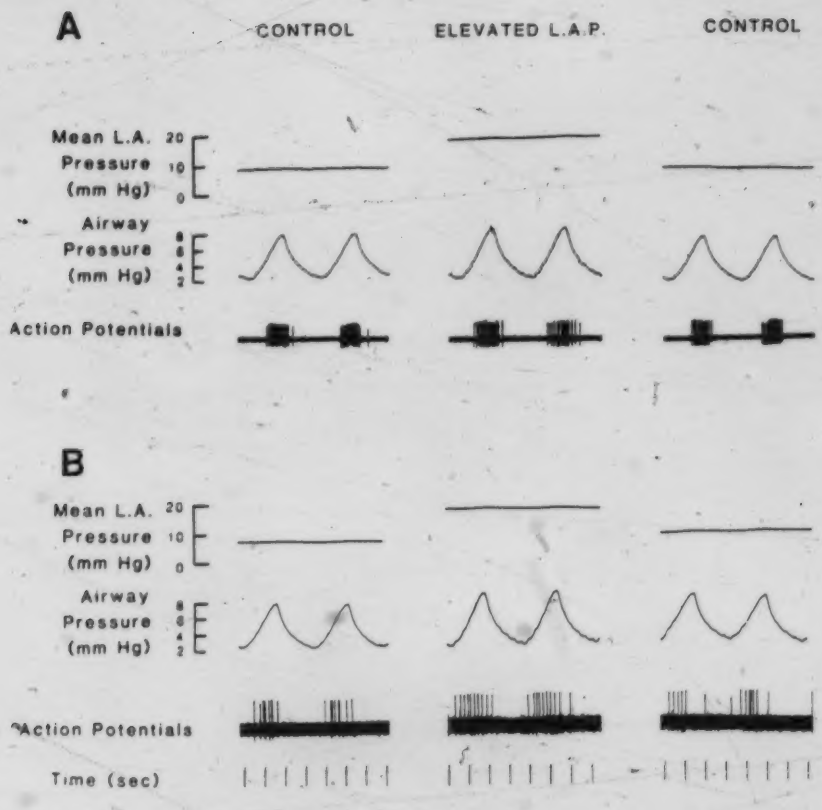

Fig. 5

Effect of an increase in left atrial (mean LA) pressure on the discharge from a SAR (A) and from a RAR (B). Tracings on the left were recorded before elevation of left atrial pressure, middle tracings 1 min after elevation and those on the right 1 min after return of left atrial pressure to control levels. The discharges from both receptors increased when the left atrial pressure was elevated.

Note: In order to enhance clarity during reproduction, some parts of the action potentials have been re-touched.

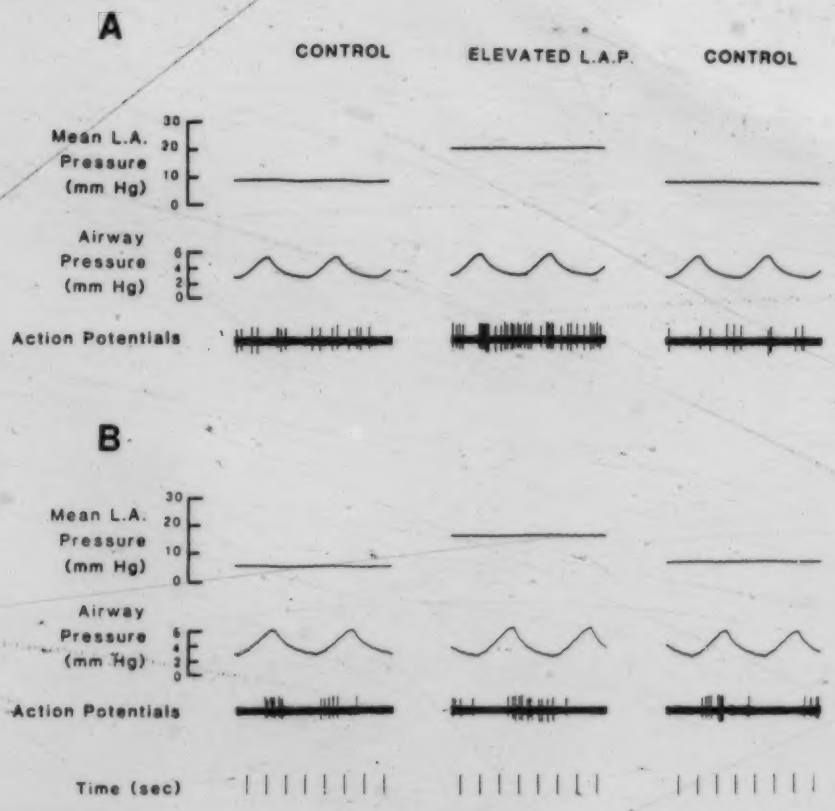

Fig. 6 Effect of an increase in left atrial (mean LA) pressure on the discharge from a bronchial C-fibre receptor (A) and a pulmonary C-fibre receptor (B). The receptor activity in these 2 units increased during elevation of left atrial pressure. Tracings on the left were recorded before elevation of left atrial pressure, middle tracings 1 min after elevation and those on the right 1 min after return of left atrial pressure to control levels.

Note: In order to enhance clarity during reproduction, some parts of the action potentials have been re-touched.

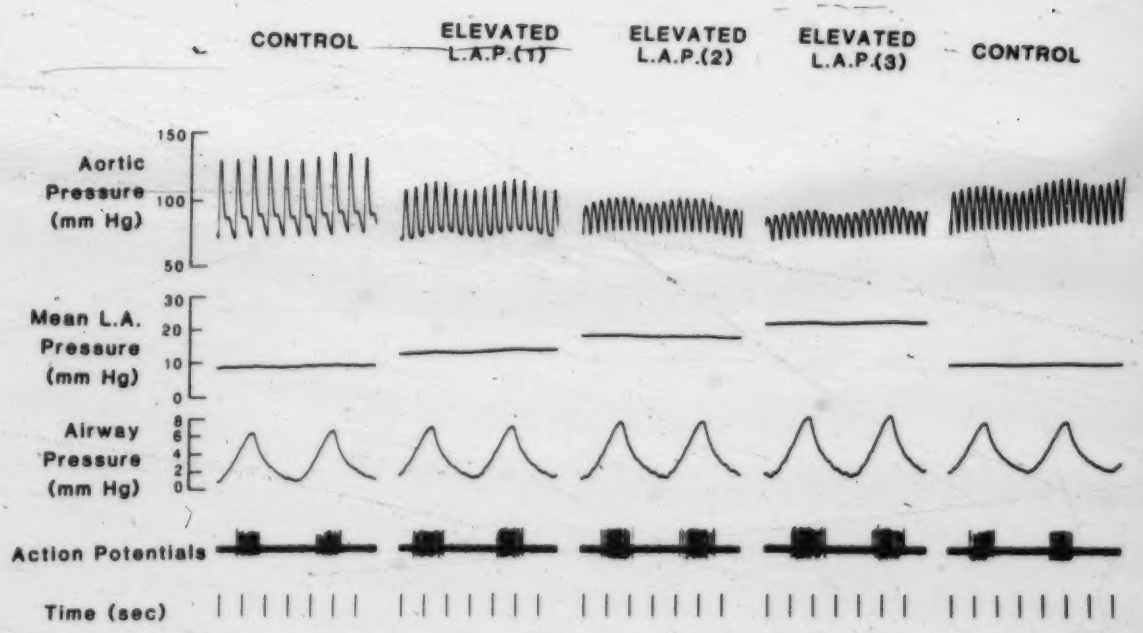

Fig. 7. An example of a SAR showing the effects of graded increases in left atrial pressure. Panels of tracings from left to right: initial control [Control], 1 min after elevation of left atrial pressure by 5 mm Hg [L.A.P.(1)], 1 min after elevation of left atrial pressure by 10 mm Hg [Elevated L.A.P.(2)], 1 min after elevation of left atrial pressure by 15 mm Hg [L.A.P.(3)] and 1 min after return of left atrial pressure to control levels [Control]. Diagram shows, from above downwards: aortic pressure (mm Hg), mean left atrial (LA) pressure (mm Hg), nerve action potentials and time (sec).

Note: In order to enhance clarity during reproduction, some parts of the action potentials have been re-touched.

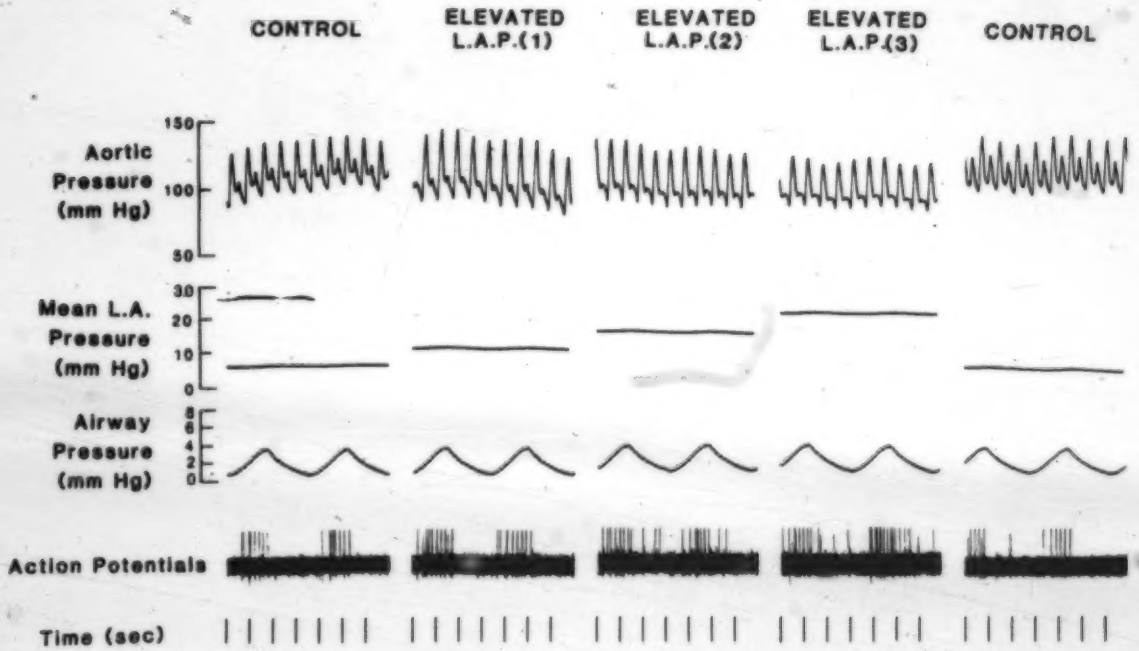

Fig. 8. An example of a RAR showing the effects of graded increases in left atrial pressure. Panels of tracings from left to right: initial control [Control], 1 min after elevation of left atrial pressure by 5 mm Hg [L.A.P.(1)], 1 min after elevation of left atrial pressure by 10 mm Hg [Elevated L.A.P.(2)], 1 min after elevation of left atrial pressure by 15 mm Hg [L.A.P.(3)] and 1 min after return of left atrial pressure to control levels [Control]. Diagram shows, from above downwards: aortic pressure (mm Hg), mean left atrial (LA) pressure (mm Hg), nerve action potentials and time (sec).

Note: In order to enhance clarity during reproduction, some parts of the action potentials have been re-touched.

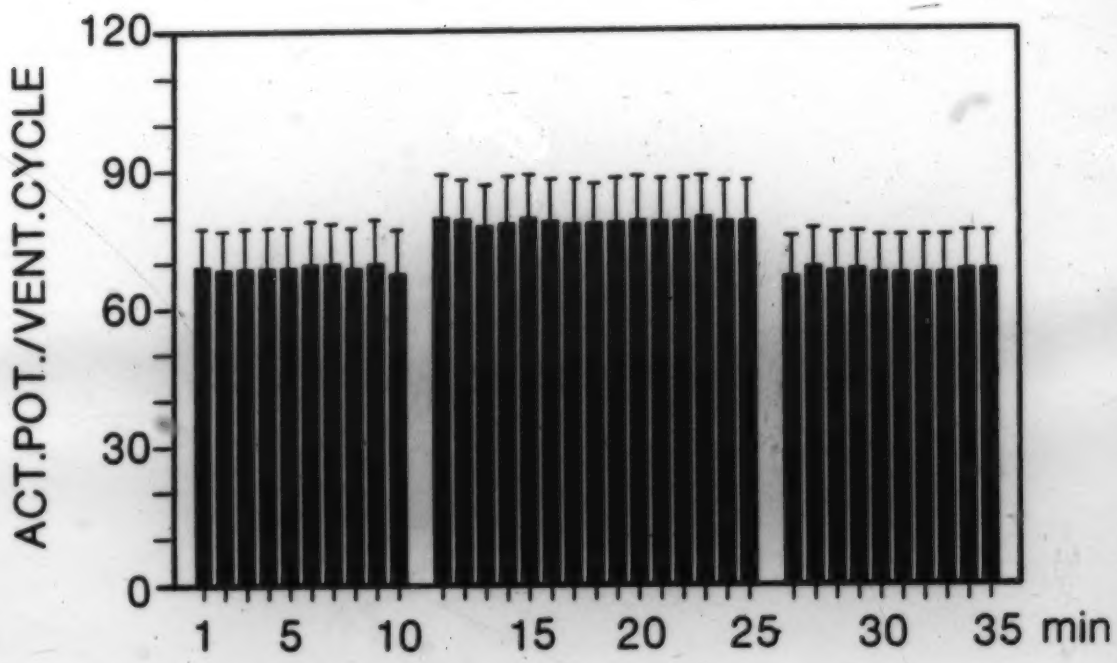

Fig. 9. Mean SAR (n = 15) activity as action potentials per ventilatory cycle (ACT.POT./VENT.CYCLE) in each minute during 10 min of initial control (1-10 min), 15 min of elevated left atrial pressure (11-25 min) and 10 min of final control (26-35 min). Bars represent +SEM.

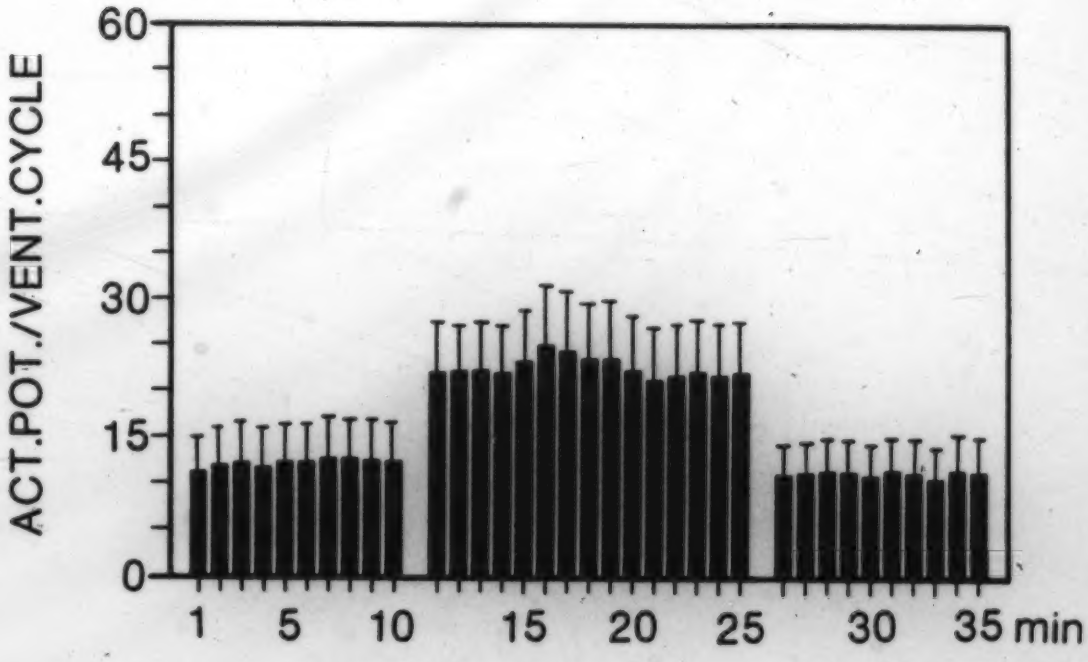

Fig. 10. Mean RAR (n = 11) activity as action potentials per ventilatory cycle (ACT.POT./VENT.CYCLE) in each minute during 10 min of initial control (1-10 min), 15 min of elevated left atrial pressure (11-25 min) and 10 min of final control (26-35 min). Bars represent +SEM.

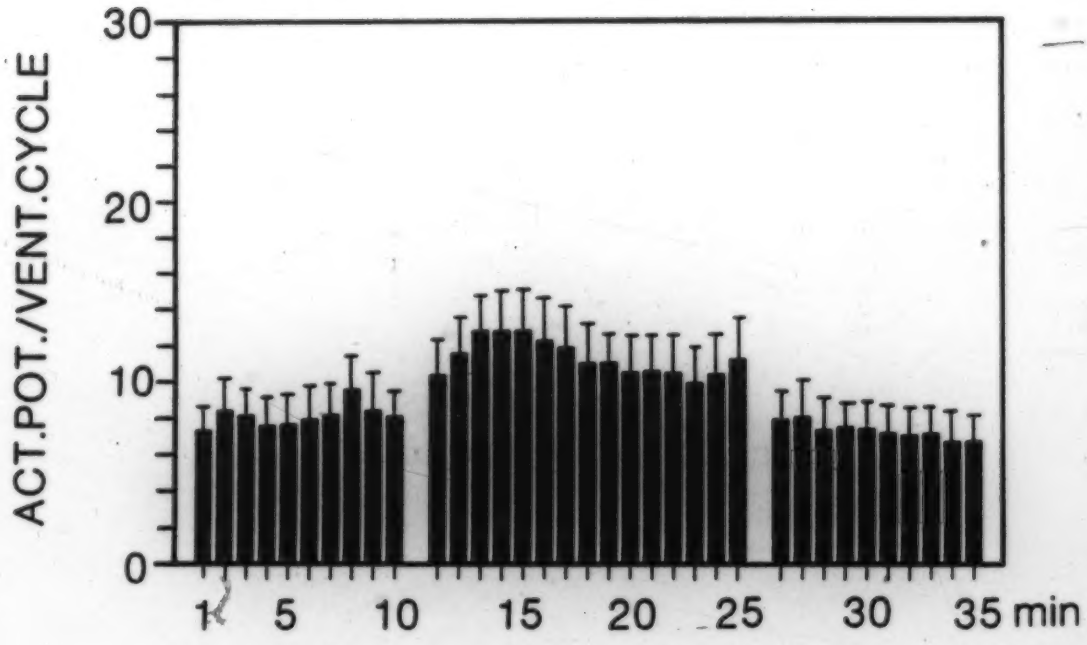

Fig. 11. Mean bronchial C-fibre receptor (n = 9) activity as action potentials per ventilatory cycle (ACT.POT./VENT.CYCLE) in each minute during 10 min of initial control (1-10 min), 15 min of elevated left atrial pressure (11-25 min) and 10 min of final control (26-35 min). Bars represent +SEM.

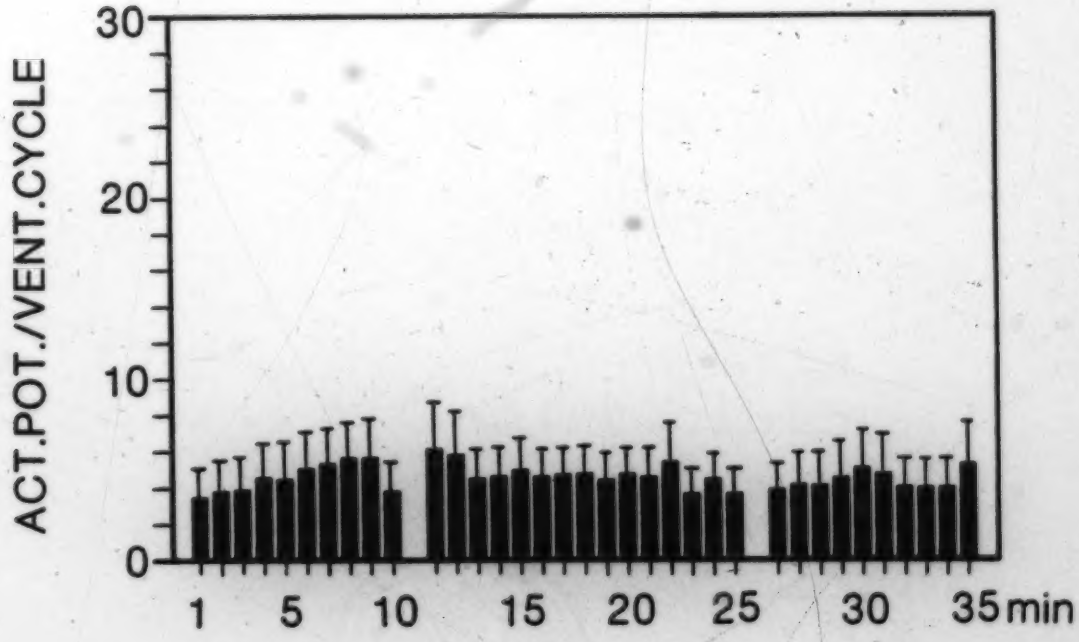

Fig. 12. Mean pulmonary C-fibre receptor (n = 9) activity as action potentials per ventilatory cycle (ACT.POT./VENT.CYCLE) in each minute during 10 min of initial control (1-10 min), 15 min of elevated left atrial pressure (11-25 min) and 10 min of final control (26-35 min). Bars represent +SEM.

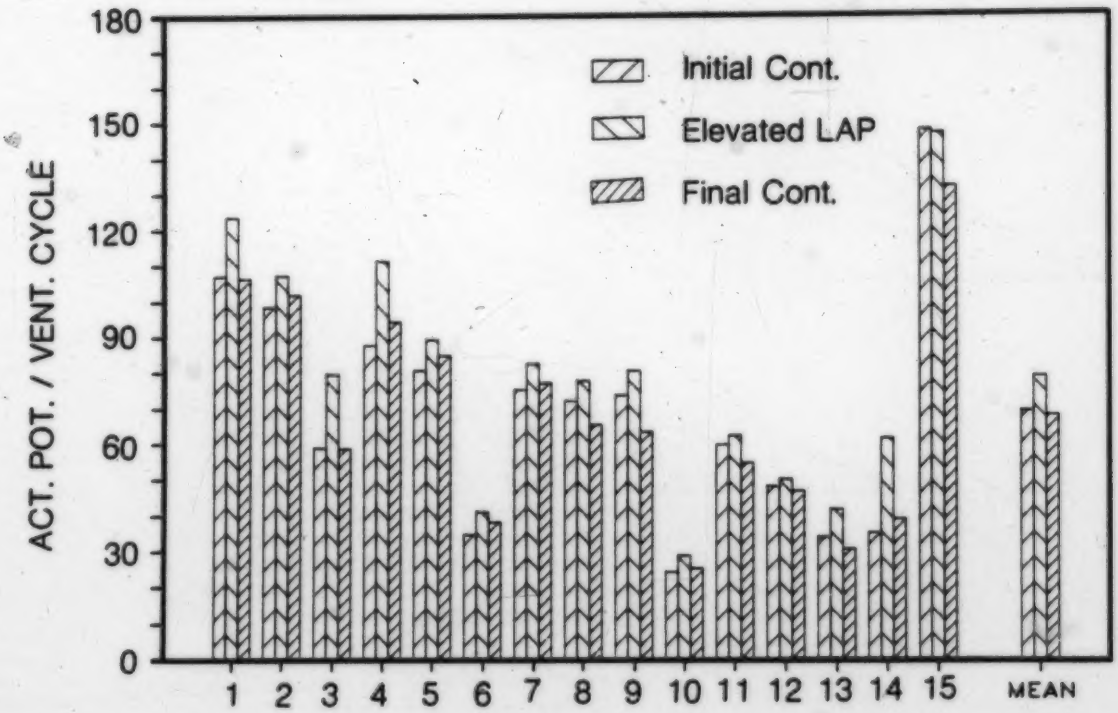

Fig. 13. Average receptor activity (ACT.POT./VENT.CYCLE) in each of the 15 SAR (1 - 15) and the mean activity of the group (MEAN) during initial control left atrial pressure for 10 min (Initial Cont.), when left atrial pressure was elevated by 10 mm Hg for 15 min (Elevated LAP) and during the final control period for 10 min (Final Cont.). SAR 1-14 showed increases in activity when the left atrial pressure was elevated when compared to initial control.

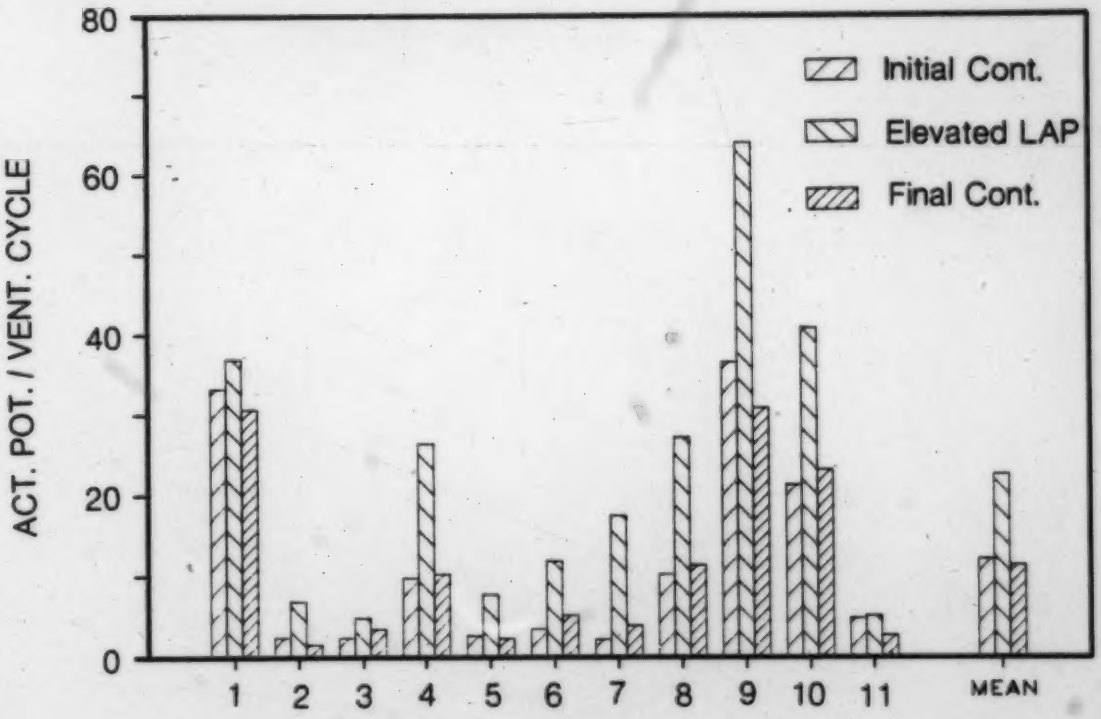

Fig. 14. Average receptor activity (ACT.POT./VENT.CYCLE) in each of the 11 RAR (1 - 11) and the mean activity of the group (MEAN) during initial control left atrial pressure for 10 min (Initial Cont.), when left atrial pressure was elevated by 10 mm Hg for 15 min (Elevated LAP) and during the final control period for 10 min (Final Cont.). All RAR showed increases in activity when the left atrial pressure was elevated when compared to controls.

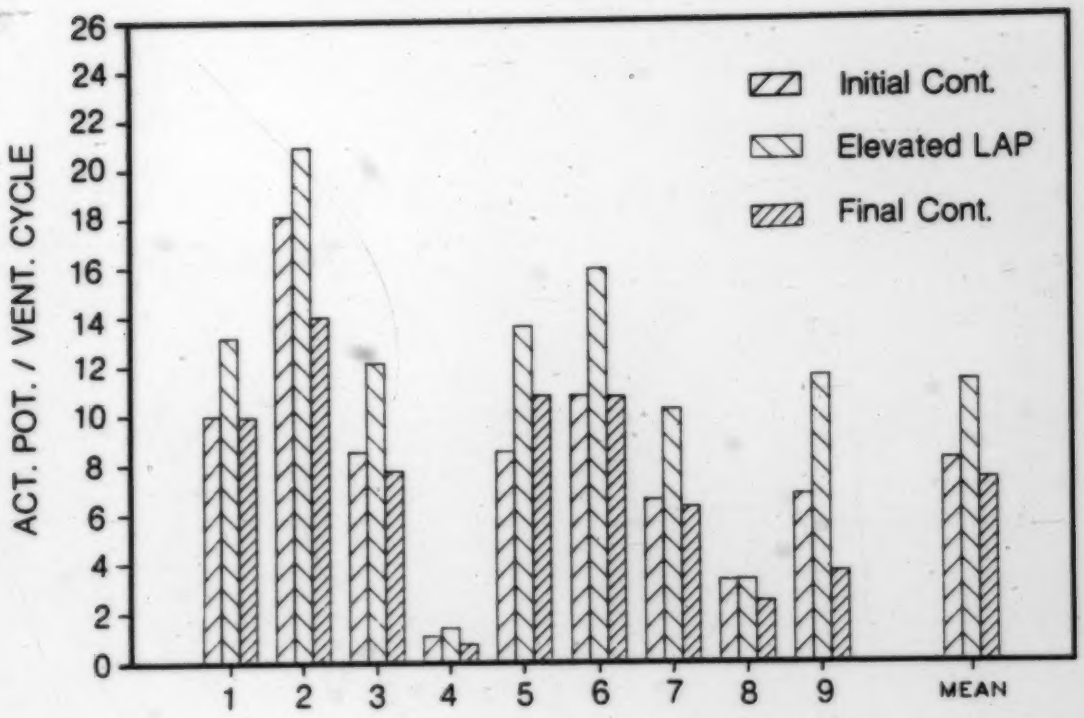

Fig. 15. Average receptor activity (ACT.POT./VENT.CYCLE) in each of the 9 bronchial C-fibre receptors (1 - 9) and the mean activity of the group (MEAN) during initial control left atrial pressure for 10 min (Initial Cont.), when left atrial pressure was elevated by 10 mm Hg for 15 min (Elevated LAP) and during the final control period for 10 min (Final Cont.). All bronchial C-fibre receptors showed increases in activity when the left atrial pressure was elevated when compared to controls.

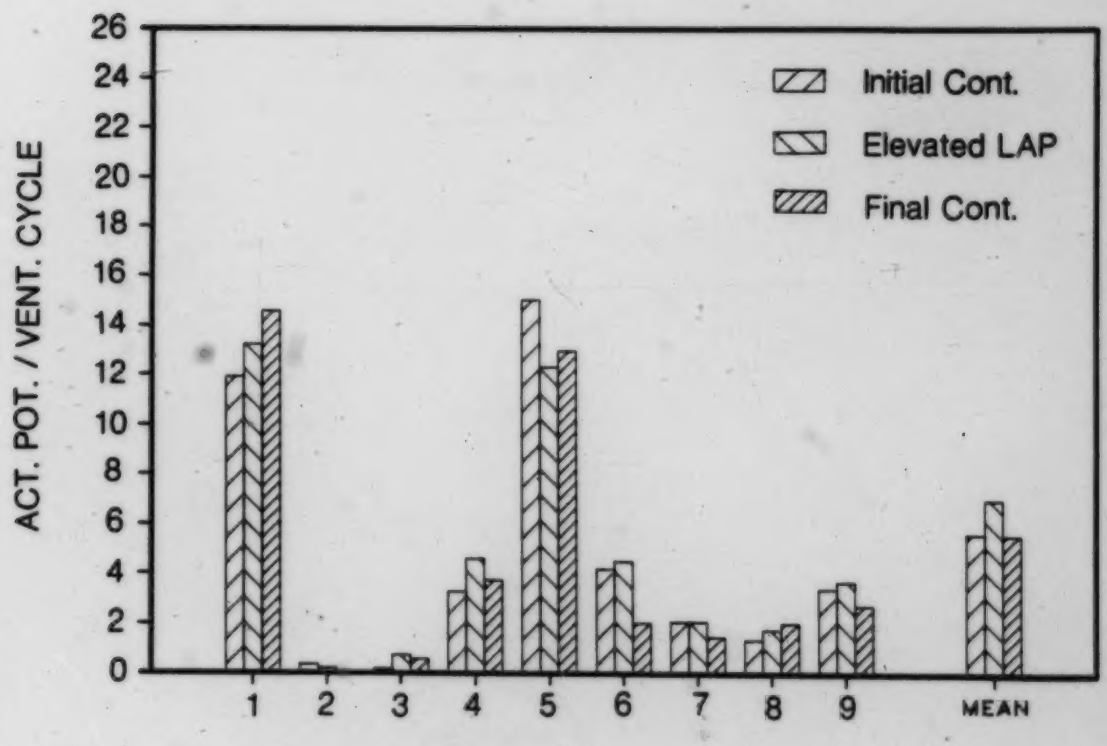

Fig. 16. Average receptor activity (ACT.POT./VENT.CYCLE) in each of the 9 pulmonary C-fibre receptors (1 - 9) and the mean activity of the group (MEAN) during initial control left atrial pressure for 10 min (Initial Cont.), when left atrial pressure was elevated by 10 mm Hg for 15 min (Elevated LAP) and during final control period for 10 min (Final Cont.). Pulmonary C-fibre receptors 1,2 and 5 did not show increases in activity when the left atrial pressure was elevated when compared to controls.

CHAPTER 9

Protocol II. The Effect of Pulmonary Venous Congestion on Pulmonary Lymphatic Flow

The experiments in this protocol were designed to study the effect of pulmonary venous congestion, produced by partial obstruction of the mitral valve, on the pulmonary lymphatic flow. The design of the protocol was based on the method reported by Uhley et al (18) who described a method of collecting lymph from those areas of the lung, i.e., the right lung and the lower lobes of the left lung, which drain into the right external jugular vein via the right lymphatic vessels, in particular, the right lymphatic duct.

After the dog had been prepared as described in the chapter on General Experimental Methods, the right external jugular vein was dissected at the base of the neck in preparation for the collection of pulmonary lymph.

Collection of Pulmonary Lymph from the Right Lymphatic Duct

Initial attempts were made to collect lymph draining into an isolated pouch made from the right external jugular vein at the base of the neck. This pouch was prepared as follows. A short segment of the right external jugular vein was isolated as shown (see Fig.17). Loose snares were placed around the axillary vein, external jugular vein, brachiocephalic vein and other venous branches which drain that region of the neck. Great care was taken not to damage the small lymphatic vessels draining into the external jugular vein (18). A long cannula (ID 1.67 mm, Intramedic polyethylene tubing) was inserted into one of the branches of the external jugular vein. This cannula was connected to a container for collecting the lymph later.

By ligating the veins draining into the external jugular vein, it was possible to create a pouch of the vein into which the lymphatic vessels drain.

Initial attempts were made to collect the lymph from this isolated venous pouch by draining it. Several problems were immediately encountered during these attempts. First and foremost was contamination due to small amounts of blood leaking into the pouch from the venous branches despite the care taken to ligate them. Clotting of the blood caused blockade of the cannula draining the pouch. Also, blood clots in the pouch impeded the free drainage of lymph into the pouch.

Therefore, a second approach was taken. The right main lymphatic duct was isolated. At the point where it entered the pouch, a small incision was made on the vessel wall and a small capillary tube (25 microliters Disposable Micro-pipets, Fisher Scientific Co., Pittsburgh, PA) was introduced into into the vessel via this incision. It was not possible to advance the capillary tube too far due to the presence of valves. The capillary tube was secured in position by suturing it to the vessel wall by fine silk ligatures. The capillary tube was positioned at a small horizontal angle and the lymph flowed out by capillary action.

It is accepted that the lymph from this right lymphatic duct did not necessarily include all the lymph from the right lung and lower lobes of the left lung. But, because the right lymphatic duct was the major lymph vessel draining this area of the lungs, it would be reasonable to expect that the amount of lymph draining and collected from it would indicate changes in the lymphatic flow in these areas

of the lungs under various conditions.

Experimental Protocol

After the above preparation was made, the animal was left to equilibrate for 5 to 10 minutes to allow the cardiovascular parameters to settle as well as to drain out any contamination in the capillary tube.

Lymph collection at baseline conditions was made for 30 minutes. Left atrial pressure was then elevated by 10 mm Hg above control level. This was maintained for 30 minutes and the lymph was collected continuously during this period. Left atrial pressure was then returned to control and lymph collection was made for another 30 minutes.

Arterial blood was obtained at the midpoint of each collection period. Plasma was collected after the blood was centrifuged. Both lymph and plasma samples were analysed for their total protein, albumin and globulin contents.

Statistical Analysis

Group data was expressed as means $\pm$ standard errors of the means. A p value < 0.05 was accepted as indicative of statistical significance. Where two or more treatments were compared by an analysis of variance, the Least Significant Difference (LSD) test at $p<0.05$ was used to detect differences between means if the ANOVA was significant (235).

The amounts of lymph collected at each of the control periods were compared with those collected during the 30 minutes of pulmonary venous congestion by an analysis of variance. The protein content of the lymph and also of the plasma taken during each of the intervals

was similarly compared. Heart rate, mean arterial pressure and left atrial pressure were compared in the same way.

RESULTS

Lymph was collected from the right lymphatic duct in four dogs. The quantity of lymph collected for 30 minutes during baseline control condition was 0.79 $\pm$ 0.27 ml. When left atrial pressure was increased from 7.6 $\pm$ 1.7 to 16.3 $\pm$ 2.7 mm Hg for 30 minutes, the volume of lymph collected was 4.05 $\pm$ 1.01 ml. Thus, there was a 5 fold increase in the volume of lymph collected during pulmonary venous congestion ($p<0.05$). This increase in the lymphatic flow was evident within one minute of raising the left atrial pressure. Following the return of left atrial pressure to control levels, the amount of lymph collected during 30 minutes was 1.63 $\pm$ 0.27 ml. This was significantly less than the quantity collected when the left atrial pressure was elevated. But, it was more than that collected during the initial control period, though the difference was not significant.

Protein concentration in the lymph during the initial control period was 2.13 $\pm$ 0.41 g/L albumin, 1.48 $\pm$ 0.22 g/L globulin and 3.61 $\pm$ 0.51 g/L total protein. The corresponding plasma protein concentration of blood taken during the initial control period was 2.81 $\pm$ 0.22 g/L albumin, 2.68 $\pm$ 0.68 g/L globulin and 5.49 $\pm$ 0.88 g/L total protein. The ratios of the protein in the lymph to that in plasma was 0.76 for albumin, 0.55 for globulin and 0.66 for total protein.

During pulmonary venous congestion, the protein contents of the lymph was 1.83 $\pm$ 0.41 g/L albumin, 0.82 $\pm$ 0.20 g/L globulin and

2.65 ± 0.52 g/L total protein. The corresponding plasma protein contents were 2.59 ± 0.46 g/L albumin, 2.36 ± 0.79 g/L globulin and 4.95 ± 1.18 g/L total protein. The lymph-plasma protein ratios were 0.71 for albumin, 0.35 for globulin and 0.54 for total protein. There was a decrease in the lymph-plasma ratio compared to the initial control ratios for all proteins. The protein contents of the lymph during the final control period were 1.65 ± 0.31 g/L albumin, 0.86 ± 0.12 g/L globulin and 2.51 ± 0.35 g/L total protein. The plasma protein for the same period were 2.38 ± 0.59 g/L albumin, 2.41 ± 0.82 g/L globulin and 4.79 ± 1.37 g/L total protein. The lymph plasma protein ratios were 0.69 for albumin, 0.36 for globulin and 0.52 for total protein.

Heart rate during the initial control period was 138 ± 7 beat/min. When left atrial pressure was elevated, it was 146 ± 7 beat/min and during the final control period it was 135 ± 8 beat/min. The corresponding mean arterial pressures were 113 ± 4 mm Hg, 97 ± 5 mm Hg and 116 ± 4 mm Hg.

The results are summarized in Table 8.

The findings of this protocol indicate that with pulmonary venous congestion following partial obstruction of the mitral valve, pulmonary lymphatic flow increased by nearly 5-fold. This finding is consistent with the hypothesis describing the stages of development of pulmonary oedema (see pg.16-17) (35). In the early stages of its development, with the increases in interstitial oedema, there is an increase in transfer of liquid and colloid from the capillaries through the interstitium due to an imbalance of factors in the Starling Equation, as found in the present study. When this occurs,

there is an equal increase in lymphatic flow due to increased lymphatic pumping. The proportion of proteins in the lymph to the plasma proteins indicate that the lymphatic fluid was a transudate. The lymphatic protein contents found in this study were similar to those reported previously in the sheep (8). The findings are also similar to previous investigations on the nature of lymphatic fluid during pulmonary congestion (8).

TABLE 8

Haemodynamic Parameters, Lymph Collected, Lymph and Plasma Protein Contents during Control and Elevation of Left Atrial Pressure

	Control	Elevated LAP	Control
Left Atrial Pressure (mm Hg)	7.6±1.7	16.3±2.7*	7.9±1.3
Heart Rate (beat/min)	138±7	146±7	135±8
Mean Arterial Pressure (mm Hg)	113±4	97±5	116±4
Lymph Collected (ml in 30 min)	0.79±0.27	4.05±1.01*	1.63±0.27
Lymph Proteins			
Albumin (g/L)	2.13±0.41	1.83±0.41	1.65±0.31
Globulin (g/L)	1.48±0.22	0.82±0.20	0.86±0.12
Total Proteins (g/L)	3.61±0.51	2.65±0.52	2.51±0.35
Plasma Proteins			
Albumin (g/L)	2.81±0.22	2.59±0.46	2.38±0.59
Globulin (g/L)	2.68±0.68	2.36±0.79	2.41±0.82
Total Proteins (g/L)	5.49±0.88	4.95±1.18	4.79±1.37
Lymph/Plasma Protein Ratio			
Albumin	0.76	0.71	0.69
Globulin	0.55	0.35	0.36
Total Proteins	0.66	0.54	0.52

Note : * $p < 0.05$

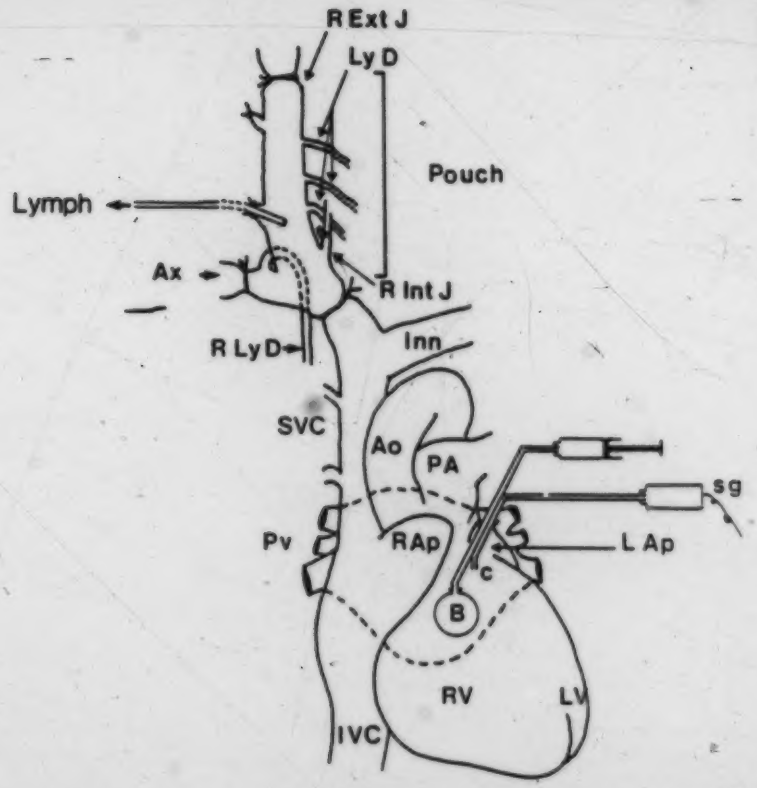

Fig. 17. Details of experimental preparation for the collection of lymph. During initial attempts, an isolated venous pouch (Pouch) was prepared from the right external jugular vein (R Ext J) by ligating it (cranially and caudally), the axillary vein (Ax), right internal jugular vein (R Int J) and other small veins draining into the pouch. The right lymphatic duct (R Ly D) and other lymphatic vessels (Ly D) were left intact. A long cannula inserted into this pouch was used to collect the lymph draining into the pouch. However because of a number of technical problems (see text), the right lymphatic duct was cannulated directly using a capillary tube and lymph flowing out of the tube was collected.

A balloon tipped cannula (B) and a pressure cannula (C) were inserted into the left atrium via the left atrial appendage (L Ap). Pressure in the left atrium was monitored by the cannula C connected to a transducer (sg). Ao = aorta, Inn = innominate vein, IVC = inferior vena cava, SVC = superior vena cava, LV = left ventricle, RV = right ventricle, R Ap = right atrial appendage, PA = pulmonary artery and Pv = pulmonary veins.

CHAPTER 10

Protocol III. The Effect of Pulmonary Venous Congestion on Dynamic Compliance

Dynamic compliance of the lungs in the open chest dog was examined during the control periods and during the period of pulmonary venous congestion produced by partial obstruction of the mitral valve. Dynamic compliance was derived from each respiratory cycle by analysing simultaneously obtained pneumotachograph generated airflow curves and intra-tracheal airway pressure curves.

Derivation of Dynamic Compliance in the Open-Chest Artificially Ventilated Dog

The dog was prepared as described in the chapter on General Experimental Methods with the following modifications to the ventilatory arrangement. The inlet and outlet connections of a pneumotachograph (Pneumotachograph Model MCI-3, Validyne Engineering Corp., Northbridge, CA) were connected to the end of the endotracheal tube and the common end of the inspiratory-expiratory lines of the constant volume ventilator respectively (Fig.18). In order to keep the lungs from collapsing at end expiration, as in the other protocols of this study, an adjustable clamp was placed at the end of of the expiratory tube of the ventilator. The clamp was adjusted such that the end expiratory pressure was similar to that when this expiratory tube was under 2 to 3 cm of water, i.e., maintaining a positive end expiratory pressure (PEEP) of 2-3 cm H_2O.

With each respiratory cycle, the pneumotachograph generated an air-flow curve so that at the end of inspiration, the area under the curve relative to time represented the tidal volume. With expiration,

the direction of flow was reversed, and the airflow curve reversed its direction. At end expiration, the area under the expiratory airflow curve in relation to time would be equal to the inspiratory airflow curve and both were equal to the tidal volume, in the steady state situation (see Fig.19). A simultaneous intra-tracheal airway pressure curve (airway pressure relative to the atmosphere) was obtained. Both curves were recorded on light sensitive paper on the Electronics for Medicine/Honeywell recording system described earlier (pg.80).

Dynamic compliance was calculated from pressure measurements which were made at the points when no gas was flowing at the end-inspiratory and end-expiratory 'no-flow' turn-round points (236). These end-inspiratory and end-expiratory points were determined on the pneumotachograph record and the airway pressure measured at these points. Dynamic compliance was calculated as follows:

$$\text{Dynamic Compliance} = \frac{\text{Change in lung volume}}{\text{Change in airway pressure}}$$

$$\text{i.e., Dynamic Compliance} = \frac{\text{Tidal Volume}}{\text{Peak Airway Pressure - End Exp. Pressure}}$$

Experimental Protocol

After the above preparation was made, the dog was left to equilibrate for 5-10 min. Then control observations were made for 5 min. Graded increases in left atrial pressure in steps of +5 mm Hg, +10 mm Hg and +15 mm Hg above the initial left atrial pressure

were made and each left for 5 min to achieve a steady state. Simultaneous recordings were made of the airflow and intra-tracheal airway pressure curves during the 5 min. Six respiratory cycles were analysed and the average obtained for the dynamic compliance of the lungs at that stage calculated.

Left atrial pressure, heart rate, mean arterial pressure, mean and peak airway pressures during these periods were noted.

Statistical Analysis

Group data was expressed as means $\pm$ standard errors of the means. A p value < 0.05 was accepted as indicative of statistical significance.

The dynamic compliance, left atrial pressure, heart rate, mean arterial pressure, mean and peak airway pressures during pulmonary venous congestion were each compared with the corresponding observations during the control periods using an analysis of variance. The differences between means were tested using a Least Significant Difference Test at $p < 0.05$ if the analysis of variance showed significant differences (235).

Results

Variations in dynamic compliance with each respiratory cycle during each stage of left atrial pressure was analysed by computing the coefficient of variation in 2 animals. In one, this was found to be 1.35% for the initial control level, 1.39% when left atrial pressure was increased by 5 mm Hg, 2.13% for 10 mm Hg, 2.77% for 15 mm Hg and 1.47% when left atrial pressure returned to the control level. For the second animal, the corresponding coefficients of variation were 4.31%, 2.33%, 4.05%, 2.94% and 1.97%.

Dynamic compliance of the lungs were studied in 5 dogs. Average dynamic compliance at initial control left atrial pressure was 69.5 ± 10.1 ml/mm Hg. At a left atrial pressure of +5 mm Hg above control it became 68.5 ± 10.4 ml/mm Hg; at +10 mm Hg, it was 63.0 ± 10.4 ml/mm Hg and at +15 mm Hg it was 60.0 ± 9.8 ml/mm Hg. During the final control period, it became 65.7 ± 9.8 ml/mm Hg. The dynamic compliance at left atrial pressures of +10 mm Hg and +15 mm Hg above control were significantly lower than the others ($p<0.05$).

Mean left atrial pressure at these stages were 5.8 ± 0.8 mm Hg, 10.2 ± 0.6 mm Hg, 14.8 ± 0.4 mm Hg, 19.7 ± 0.6 mm Hg and 5.8 ± 0.8 mm Hg. The corresponding heart rates at these stages were 127 ± 7 beat/min, 138 ± 8 beat/min, 149 ± 4 beat/min, 153 ± 8 beat/min and 131 ± 7 beat/min. The corresponding mean arterial pressures were 116 ± 2 mm Hg, 113 ± 1 mm Hg, 109 ± 1 mm Hg, 103 ± 2 mm Hg and 118 ± 1 mm Hg.

The results are summarized in Table 9.

The experiments in this protocol measuring the dynamic compliance of the lungs indicate that there was a small but significant decrease in the dynamic compliance of the lungs during pulmonary venous congestion. A graded decrease in dynamic compliance was noted during stepwise increases in left atrial pressure. The magnitude and degree of change of the dynamic compliance during pulmonary congestion found in this study are compatible with that shown by Jones et al (6). These authors reported a resting dynamic compliance of about 30 ml/cm H_2O which decreased to approximately 20 ml/cm H_2O when pulmonary capillary wedge (i.e. left atrial) pressure increased by 12 mm Hg in the closed chest dog. In this earlier study, the dynamic compliance

involved both the chest wall and lungs, whereas that of the present study involved the lungs only. This explains why a higher value for dynamic compliance was obtained here.

The suggestion that these changes were due to the local effects on interstitial oedema "stiffening" the lungs and causing narrowing of the small airways so that a greater trans-airway pressure was required to expand a given volume of lung is a speculation that seems reasonable but that needs further study. However, in the study referred to in the preceding paragragh, using methods almost similar to that of the present study in causing partial obstruction of the mitral valve, Jones et al (6), reported a significant increase in closing volumes of the lungs when the left atrial pressure was increased by about 10 mm Hg. The term closing volume is a measure of lung volume that is reached at which the airways at the base of the lungs begin to close and it is thought that increases in closing volumes indicate small airway disease. It has been suggested that these changes in closing volumes were due to a vagal-mediated increase in resistance of the small airways rather than compression of these airways by oedema fluid (6).

TABLE 9

Cardiorespiratory Parameters during Periods of Control and Graded Increases in Left Atrial Pressure during Studies on Dynamic Compliance of the Lungs (n = 5)

	INCREASES IN LEFT ATRIAL PRESSURE				
	Control	5 mm Hg	10 mm Hg	15mm Hg	Control
LAP (mmHg)	$^{a}5.8\pm0.8$	$^{b}10.2\pm0.6$	$^{c}14.8\pm0.4$	$^{d}19.7\pm0.6$	$^{a}5.8\pm0.8$
HR (b/m)	$^{a}127\pm7$	$^{a}138\pm8$	$^{bc}149\pm4$	$^{c}153\pm8$	$^{a}131\pm7$
MAP (mmHg)	$^{a}116\pm2$	$^{a}113\pm1$	$^{b}109\pm1$	$^{b}103\pm4$	$^{a}118\pm1$
Mean ITAP (mmHg)	$^{a}3.41\pm0.27$	$^{a}3.45\pm0.28$	$^{a}3.50\pm0.27$	$^{a}3.51\pm0.25$	$^{a}3.45\pm0.27$
Peak ITAP (mmHg)	$^{a}5.56\pm0.41$	$^{a}5.70\pm0.50$	$^{b}6.00\pm0.61$	$^{b}6.12\pm0.62$	$^{a}5.74\pm0.44$
Dyn.Comp. (ml/mm Hg)	$^{a}69.5\pm10.1$	$^{ab}68.5\pm10.4$	$^{c}63.0\pm10.4$	$^{d}60.0\pm9.8$	$^{b}65.7\pm9.8$

Note: In each parameter, values with different prefixes are significantly different from each other ($p<0.05$)

LAP: Left Atrial Pressure; HR (b/m): Heart Rate (beat/min);
MAP: Mean Arterial Pressure; ITA : Intra-tracheal Airway Pressure.
Dyn.Comp.: Dynamic Compliance of the Lungs.

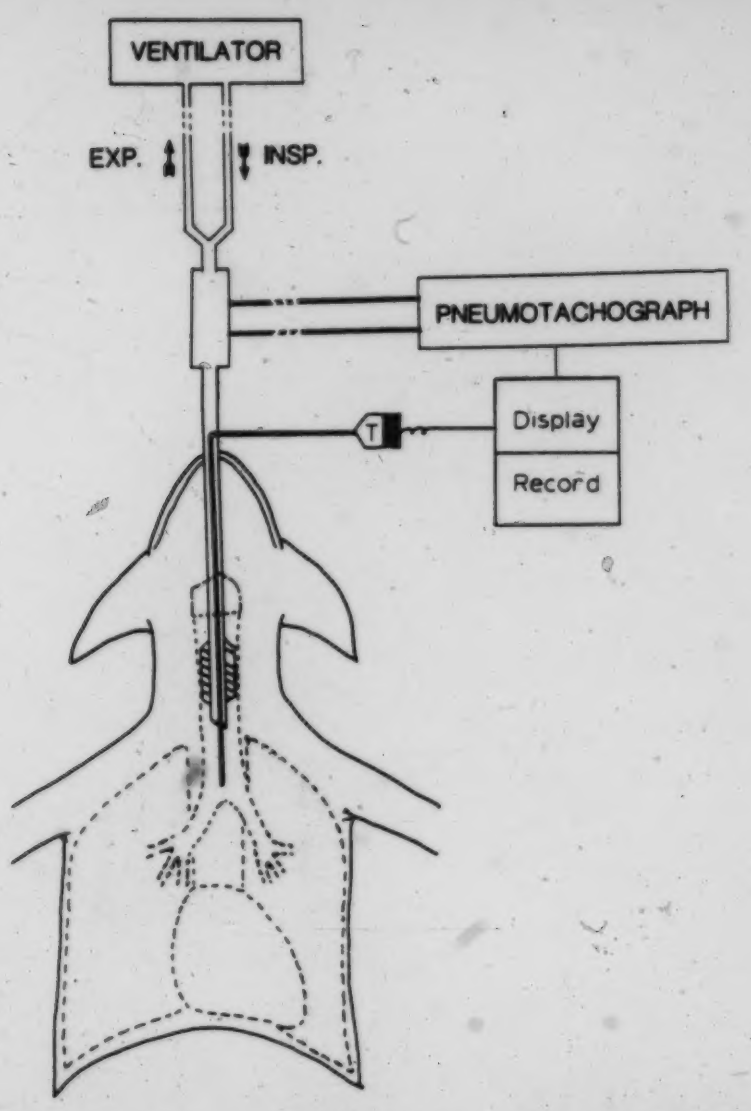

Fig. 18. Experimental preparation for the measurement of dynamic compliance of the lungs. The animal was ventilated by IPPV using a constant volume ventilator via a cuffed endotracheal tube. Intra-tracheal airway pressure at the level of the carina was monitored by a cannula connected to a transducer (T) as shown. The pneumotachograph was connected to the endotracheal tube and the common tube joining the inspiratory (INSP.) and expiratory (EXP.) lines of the ventilator. The air-flow curve generated by the pneumotachograph with each ventilatory cycle and the simultaneous intra-tracheal airway pressure curve were displayed on a multichannel oscilloscope and recorded on light sensitive paper (see Fig.19).

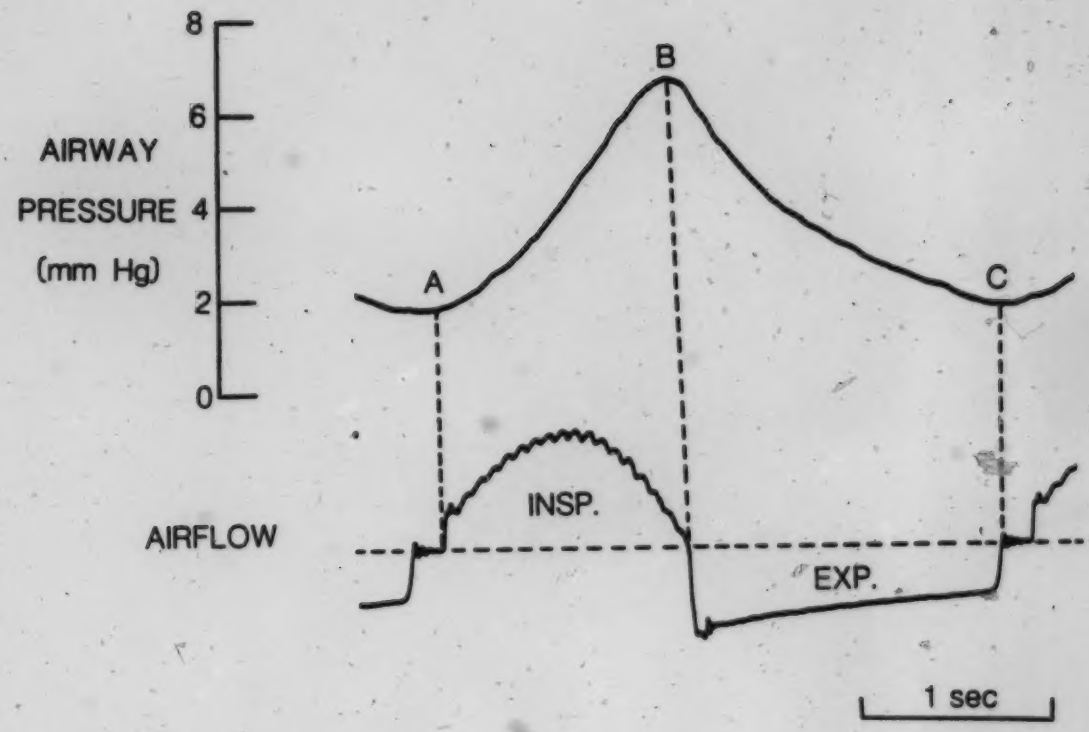

Fig. 19. An example of intra-tracheal airway pressure and air-flow curves obtained simultaneously. The areas bounded by the inspiratory (INSP.) and expiratory (EXP.) portions of the airflow curve were equal and were proportional to the tidal volume. There was no air-flow at points A and C as these represented beginning of inspiration and end expiration respectively. At point B, end inspiration, there was also no air-flow. Intra-tracheal airway pressures were measured at these point. Dynamic compliance of the lungs was calculated as follows: tidal volume/ (pressure at B - average of pressures at A and C). See text for discussion.

CHAPTER 11

Protocol IV. The Changes in Tone of a Tracheal Segment during Pulmonary Venous Congestion

The series of experiments in this section investigated the effects of pulmonary venous congestion on an upper tracheal segment with an intact motor innervation from the superior laryngeal nerves. This method has been referred to in the section on the Literature Review dealing with measurement of changes in tone of the airway smooth muscle (pg.65-66). Additional manoeuvres were carried out to establish the possible mechanisms involved in the observed changes in tracheal tone during pulmonary venous congestion.

Following preparation of the animal as described in the chapter on General Experimental Methods, the following additional surgery specific to this protocol was performed before observations were made.

Tracheostomy

The cervical trachea was exposed by a ventral incision and a tracheostomy was made at its lower end close to the base of the neck. A tracheostomy tube (ID 9.0 mm, Portex Tube, Smith Industries Inc., Scarborough, Ontario) was inserted and secured tightly. The endotracheal tube introduced earlier was removed. The animal was ventilated at the rate of 12-15/min and with a tidal volume of 10-12 ml/kg. The tidal volume was reduced to accommodate for the reduced anatomical dead space. Airway pressure was monitored at the level of the carina by incorporating the cannula with multiple side holes described earlier (pg.78-79) into the tracheostomy tube and connecting the other end of it to a pressure transducer. Arterial

blood gases were maintained within normal limits by adjusting the ventilator. Non-respiratory acidaemia was prevented by an infusion of sodium bicarbonate (7.5% w/v) given at the rate of 1 ml/10 min.

Maintenance of Mean Aortic Pressure

As the tracheal segment has been shown to respond to stimulation of peripheral baroreceptors due to changes in aortic pressure (222,223), care was taken in this protocol to avoid sudden and excessive falls in mean aortic pressure during production of pulmonary venous congestion. A septostomy catheter was introduced into the upper abdominal aorta. This septostomy catheter (size 5F, Fogarty Dilation Catheter; Edwards Laboratories, Santa Ana, California) was inserted through the left omocervical artery at the base of the neck and advanced into the descending aorta such that the balloon at the tip of this catheter lay caudal to the tip of the cannula used for monitoring aortic pressure. Inflation of this balloon increased pressure in the aorta cranial to the balloon.

Measurement of Tracheal Smooth Muscle Tension

Isometric tension was recorded in vivo in an upper tracheal segment by a force-displacement transducer according to the method described by Brown et al (200). After exposure of the cervical trachea and retraction of the overlying neck muscles, an upper segment of 5-6 cartilaginous rings was incised ventrally in the midline and then tranversely at both ends around the cartilaginous rings down to the posterior muscular wall which was left intact. The cut edges were retracted laterally and each was attached to a light plastic bar by nylon sutures. The bar on the right of the animal was anchored to a fixed metal bar so that this tracheal segment was in

the same horizontal plane as the posterior trachealis muscle. Care was taken to avoid excessive tension from the surrounding tissues. The left tracheal segment was attached in the same manner to a force-displacement transducer (Model FT03C, Grass Medical Instruments, Quincy, Massachusetts) which was mounted on a rack and pinnion. The tracheal segments were stretched to produce a baseline tension between 50 to 100 g by adjusting the rack and pinnion. During dissection, the superior laryngeal, recurrent laryngeal and pararecurrent laryngeal nerves, which form the motor innervation to this tracheal segment, were identified. The recurrent and pararecurrent laryngeal nerves were sectioned at the beginning of the experiment so that the sole nerve supply to the tracheal segment was from the superior laryngeal nerves. The experimental preparation described here is as shown in Fig.20.

The output of the force-displacement transducer was amplified and recorded on a Gould amplifying and recording system (Model 2400S, Gould Inc., Recording Systems Division, Cleveland, Ohio). During these experiments, signals from the Electronics for Medicine recording system were fed into the Gould system so that atrial, aortic and intra-tracheal airway pressures were recorded on the same system as the tracheal tension in order to establish the temporal relationship between the various changes.

Experimental Protocols

After the preparation was completed, the animal was left for 30-60 min for equilibration. Saline at body temperature was dripped on to the exposed posterior trachealis muscle to keep it moist and prevent excessive cooling. Reactivity of the tracheal preparation

was tested by discontinuing artificial ventilation for up to one minute. As a result of hypoxia, the tracheal preparation would respond by contracting if it was viable.

Following this test, one of the following protocols was completed.

Protocol IVA. Effect of Pulmonary Venous Congestion on Tracheal Tone

A continuous recording was made during this protocol at a paper speed of 0.1 mm/s. An initial control observation of baseline tracheal tension, left atrial, aortic and intra-tracheal pressures and heart rate was made for 5 min. Then the balloon in the left atrium was inflated to raise the left atrial pressure by 10 mm Hg and recordings were continued for 5 min. At the end of this period, the balloon was deflated and a second control recording was made for 5 min. The mean pressure in the thoracic aorta was prevented from falling by inflating the septostomy balloon in the aorta.

After recording the responses as described above, in five dogs, the vagi were cooled gradually to 8°C and protocol IVA was repeated. In 3 other dogs, the effect of stimulation was studied after bilateral vagotomy. The method of vagal cooling is similar to that described under Protocol IC (pg.87-88).

As the cooling platforms were positioned next to the tracheal segment, care was taken during the experiment to prevent unintentional cooling of the segment by insulating it from the cooling platforms by pieces of styrofoam.

Both vagal cooling and vagotomy in the neck were carried out caudal to the tracheal segment preparation. This was undertaken to

examine the effects of reversible (by vagal cooling to 8°C) and irreversible (by vagotomy) blockade of the vagal afferents on the tracheal tension.

In three dogs, when the vagi were at 8°C, the effect of capsaicin (10 ug/kg; capsaicine natural, Fluka AG, Chemische Fabrik, Switzerland) injected into the right atrium was examined both when the left atrial pressure was at control value and when it was elevated by 10 mm Hg. This procedure was repeated with the vagi cooled to 1°C. This protocol was carried out to study the effect of stimulation of the C-fibre receptors on tracheal tone following blockade of the myelinated afferents.

Protocol IVB. Effect of Propranolol on Tracheal Tension during Pulmonary Venous Congestion

After Protocol IVA was completed, propranolol (0.5 mg/kg; propranolol hydrochloride, Ayerst Laboratories) was administered into the right atrium and the procedure was repeated following 10 min of re-equilibration. This protocol was undertaken to examine the effect of beta-adrenergic receptor blockade on the tracheal tone during pulmonary venous congestion.

Protocol IVC. Effect of Atropine on Tracheal Tension during Pulmonary Venous Congestion

In order to find out whether the efferent link in changing tracheal tension during pulmonary venous congestion is via a cholinergic innervation, atropine (3 mg, atropine sulfate, Squibb Canada Inc., Montreal) was injected into the right atrium following completion of Protocol IVA. After 5 to 10 min of re-equilibration, observations were made under Protocol IVA, at body temperature.

Protocol IVD. Effect of Stimulation of Left Atrial Receptors on Tracheal Tension

This protocol tested the hypothesis that changes in tracheal tension might be due to stimulation of afferent endings situated in the left atrium because, when left atrial pressure was raised, these receptors would be stimulated.

The technique of stimulating the left atrial receptors without producing a change in left atrial pressure and inducing pulmonary venous congestion was adapted from that described by Koizumi et al (169). This was done by stretching the three pulmonary vein-left atrial junctions on the left side of the heart without impeding venous flow into the left atrium or obstructing the emptying of the left atrium into the left ventricle.

Stimulation of Left Atrial Receptors

The left side of the heart was exposed by a left thoracotomy. The pericardium at the left atrial appendage was excised dorsally to the point where the left pulmonary veins join the left side of the left atrium. Two suture threads were inserted at each side of the 3 pulmonary vein-left atrial junctions. The sutures, which were approximately 120^{o} to each other in a ventral direction, together with the anchoring provided by the pericardium dorsally acted in three equi-angular directions. Thus, when weights were attached to the sutures by a pulley system, the pulmonary vein-left atrial junctions were stretched without occluding pulmonary venous return. Stimulation of the left atrial receptors were indicated by an increase in heart rate. The pressure in the left atrium was monitored by the cannula inserted through the atrial appendage (Fig. 21).

An initial preliminary study was performed to test the effectiveness of this technique. In four dogs, the pulmonary vein-left atrial junctions were stretched. The control heart rate was 89.3 $\pm$ 5.0 beat/min. When the pulmonary vein-left atrial junctions were stretched, the heart rate became 103.5 $\pm$ 3.7 beat/min, i.e., an increase in heart rate of 14.2 $\pm$ 1.2 beat/min. Following the administration of intravenous propranolol, it was found that there was no change in heart rate when the procedure was repeated (85.2 $\pm$ 3.2 beat/min before and 87.6 $\pm$ 2.8 beat/min after). This manoeuvre suggested that the increase in heart rate was reflex in nature, due to stimulation of the atrial receptors situated at the pulmonary vein-left atrial junctions.

Following completion of Protocol IVA, the left atrial receptors were stimulated without changes in left atrial pressure. The stimulus was adjusted such that an increase in heart rate comparable to that achieved by partial obstruction of the mitral valve was achieved (Protocol IVA). An initial control recording was made over 5 min. The left atrial receptors were stimulated for 5 min during which further recordings were made. The stimulus was then removed and a final control recording was made for 5 min.

Protocol IVE. Effect of Partial Obstruction of the Tricuspid Valve on Tracheal Tension

As tracheal tension increased with partial obstruction of the mitral valve, the effect of partial obstruction of the tricuspid valve and the resulting elevation of right atrial pressure on tracheal tension was studied. There was no pulmonary venous congestion during partial obstruction of the tricuspid valve but

atrial receptors in the right atrium were stimulated when the right atrial pressure was elevated.

In this protocol, a cannula (ID 1.67 mm, Intramedic polyethylene tubing) bearing a balloon at one end and another cannula (ID 1.67 mm, Intramedic polyethylene tubing) were inserted into the right atiurm via the right atrial appendage in the same manner as that performed in the left atrium. Partial obstruction of the tricuspid valve was produced by distension of the balloon in the right atrium (Fig.23). The degree of balloon distension was adjusted to produce an increase in heart rate similar to that produced by obstruction of the mitral valve in Protocol IVA. Recordings were then made as before (Fig. 22).

Protocol IVF. Effect of Stimulation of Right Atrial Receptors on Tracheal Tension

This protocol was similar to Protocol IVD and was designed to examine whether stimulation of the right atrial receptors without changing the right atrial pressure would affect the tracheal tension.

Stimulation of Right Atrial Receptors

Stimulation of right atrial receptors without producing changes in right atrial pressure was achieved by introducing an expansile cannula into the superior vena cava-right atrial junction via the external jugular vein in the neck. This metal cannula held a piston connected to some springs at one end. When the piston was pushed in, the springs expanded outwards and stretched the tissue surrounding them without impeding venous return (168) (Fig.23). Activation of the atrial receptors was indicated by an increase in heart rate. The pressure in the right atrium was monitored using a cannula, the tip of which was positioned in that chamber.

The technique used in this protocol has been described previously (168). In this earlier study, it was found that stimulating the right atrial receptors using the expansile cannula positioned at the superior vena cava-right atrial junction resulted in an increase in heart rate of 14.6 $\pm$ 2.0 (n=23). This change in heart rate was similar to that found at the preliminary study in Protocol IVD, when the left atrial receptors were stimulated by stretching the pulmonary vein-left atrial junctions on the left side.

In five dogs, the right atrial receptors at the superior vena cava-right atrial junction were stimulated without change in right atrial pressure. This was done by stretching the superior vena cava-right atrial junction for 5 min using the expansile cannula positioned at this site. The degree of stimulation was such that it produced an increase in heart rate which was similar to that produced with Protocol IVA. Recordings were made as before.

Protocol IVG. Effect of Sectioning the Superior Laryngeal Nerves during Pulmonary Venous Congestion

In three dogs, following the completion of the first part of the observation in Protocol IVA, the superior laryngeal nerves were exposed bilaterally and sectioned. Then after allowing 10 minutes for re-equilibration, control observations were made, to be followed by raising of the left atrial pressure as in Protocol IVA.

STATISTICAL ANALYSIS

Group data was expressed as means $\pm$ standard errors of the means. In all cases, a p value < 0.05 was accepted as indicative of statistical significance.

In each protocol, the data obtained during the initial and final

control periods were pooled. The data obtained during the experimental period was then compared with this pooled data using a paired t-test.

Data on tracheal tension, mean aortic, mean left atrial, and mean intra-tracheal pressures were analysed in this same way.

RESULTS

Protocol IVA. Effect of Pulmonary Venous Congestion on Tracheal Tone

Protocol IVA was completed in 17 dogs in which 53 observations were made. The pH, pO_2, pCO_2 and $[HCO_3^-]$ of the arterial blood during the control periods were 7.34 $\pm$ 0.02, 250.9 $\pm$ 20.7 mm Hg, 38.7 $\pm$ 2.4 mm Hg and 17.8 $\pm$ 0.7 mmol/L respectively. When left atrial pressure was elevated, the corresponding values were 7.29 $\pm$ 0.01, 238.4 $\pm$ 29.7 mm Hg, 44.4 $\pm$ 2.3 mm Hg and 18.7 $\pm$ 0.8 mmol/L. There were no significant differences in these arterial blood parameters measured.

The average length of the tracheal segment under investigation was 2.8 $\pm$ 0.2 cm. Average baseline tracheal tension was 91.8 $\pm$ 2.1 g. Tension increased significantly to 98.3 $\pm$ 2.2 g ($p<0.001$), i.e. an increase of 6.5 $\pm$ 0.6 g, when left atrial pressure was raised by 10.7 $\pm$ 0.2 mm Hg from a control level of 4.6 $\pm$ 0.6 mm Hg to 15.3 $\pm$ 0.6 mm hg ($p<0.001$). The raised left atrial pressure was maintained for 316 $\pm$ 17 s. Tracheal contraction began 27$\pm$3s after left atrial pressure was elevated. In most preparations ($n=41$), the maximum tension was rapidly attained and this was maintained for the duration of the stimulation. In others ($n=12$), there was a

delay in achieving the maximum tension which was attained 2 to 3 min after the preparation commenced contracting. The tracheal segment began to relax 26 ± 4 s after left atrial pressure was restored to control levels and then gradually returned to baseline over the next 2 to 3 min. An example is shown in Fig. 24.

Control mean intra-tracheal pressure was 2.23 ± 0.19 mm Hg and was 2.55 ± 0.17 mm Hg when left atrial pressure was elevated. Control peak intra-tracheal pressure was 5.29 ± 0.17 mm Hg and when left atrial pressure was raised, it was 5.69 ± 0.18 mm Hg (both, $p<0.05$).

Mean aortic pressure during control was 122 ± 3 mm Hg. When left atrial pressure was elevated, mean aortic pressure was 121 ± 2 mm Hg. There was no statistically significant difference between these two pressures. Heart rate increased significantly from 139 ± 10 beat/min to 181 ± 10 beat/min ($p<0.01$) during the manoeuvre (Table 10).

Effect of Vagal Cooling

In five dogs, following the initial observations under Protocol IVA, the cervical vagi were cooled gradually to 7 to 8°C. At 8°C, baseline tracheal tension was 91.4 ± 1.5 g. This did not change when left atrial pressure was increased from 5.20 ± 0.43 mm Hg to 15.50 ± 1.25 mm Hg. Mean arterial pressure was 132.8 ± 10.0 mm Hg during the control period and 132.0 ± 8.0 mm Hg ($p>0.05$) during elevation of left atrial pressure while heart rate changed from 162 ± 11 beat/min to 168 ± 6 beat/min ($p>0.05$). The corresponding values for mean intra-tracheal pressures were 2.30 ± 0.27 mm Hg and 2.44 ± 0.28 mm Hg respectively. The control peak intra-tracheal

pressure was 5.22 ± 0.29 mm Hg and when left atrial pressure was elevated, it became 5.44 ± 0.25 mm Hg (Table 11).

In all five dogs, an increase in tracheal tension was evident during pulmonary venous congestion after the vagi were rewarmed to body temperature. An example is shown in Fig. 25

In three dogs, the effect of the stimulus was tested after sectioning the vagi bilaterally at the points of cooling. It was found that the tracheal segment failed to contract during pulmonary venous congestion.

Effect of Capsaicin

In two dogs, capsaicin (10 ug/kg) was injected into the right atrium when the vagi were cooled to 8°C (5 observations) and when the vagi was cooled to 1°C (4 observations). At 8°C, the baseline tracheal tension was 92.0 ± 2.1 g. Following the administration of capsaicin, the tracheal segment began to contract 27.2 ± 4.5 s later and reached a maximum tension of 108.1 ± 3.5 g (an increase of 16.0 ± 1.8 g). This increase in tracheal tension was transient and lasted 76.4 ± 2.8 s. The response was observed both when left atrial pressure was at control level and when left atrial pressure was elevated with no significant differences between observations under the two conditions.

When the vagi were at 1°C, the basal tension was 90.0 ± 0 g and it increased to 97.5 ± 0.5 g, an increase of 7.5 g. This increase was significantly lower than that at 8°C ($p<0.05$). An example is shown in Fig. 26. These observations suggested that the effects of capsaicin on the tracheal segment were (a) acting directly on the tracheal smooth muscle, and (b) indirectly via stimulation of

the C-fibre receptors.

Protocol IVB. Effect of Propranolol on Tracheal Tension during Pulmonary Venous Congestion

Four dogs were studied. Baseline tracheal tension before the administration of propranolol was 101.3 ± 4.1 g and this remained unchanged (101.3±4.1 g) following the administration of propranolol (0.5 mg/kg, i.v.). The heart rate before propranolol was 155.0 ± 14.0 beat/min and after propranolol was 104.2 ± 16.2 beat/min while the arterial pressure was 145.7 ± 12.5 and 141.7 ± 11.7 mm Hg respectively.

Ten min after administration of propranolol, control tracheal tension was 101.3 ± 4.1 g. This increased to 109.0 ± 6.6 g, i.e., an increase of 7.7 ± 0.7 g ($p<0.05$), when left atrial pressure was elevated from 5.25 ± 1.9 mm Hg to 15.50 ± 5.50 mm Hg ($p<0.001$). Control mean aortic pressure and heart rate were 141.7 ± 11.7 mm Hg and 104.2 ± 16.2 beat/min. When left atrial pressure was elevated, mean aortic pressure was 141.3 ± 12.1 mm Hg and heart rate was 108.3 ± 19.2 beat/min. During the period when left atrial pressure was elevated, mean aortic pressure was partly maintained by inflating the balloon on the septostomy catheter in the descending aorta (Table 12).

Protocol IVC. Effect of Atropine on Tracheal Tension during Pulmonary Venous Congestion

Atropine (3.0 mg) was injected into the right atrium in four dogs. Before atropine, resting tracheal tension was 97.7 ± 3.9 g and this remained unchanged following administration of atropine, i.e., remaining at 97.7 ± 3.9 g. Heart rate was 109.3±20.2

beat/min and mean aortic pressure was 138.0 ± 14.9 mm Hg before atropine was injected. Ten minutes after administration of atropine, heart rate was 131.5 ± 15.2 beat/min and mean aortic pressure was 135.1 ± 10.2 mm Hg.

Left atrial pressure was raised from 5.8 ± 3.9 mm Hg to 14.5 ± 3.5 mm Hg ($p<0.01$) 10 min after the administration of atropine. Tracheal tension was 97.7 ± 3.9 g during control and this did not change when left atrial pressure was elevated. Heart rate before elevation of left atrial pressure was 131.5 ± 15.2 and mean aortic pressure was 135.1 ± 10.2. When left atrial pressure was elevated, they were 154.3 ± 7.0 beat/min and 143.3 ± 10.9 mm Hg, respectively (Table 13).

Protocol IVD. Effect of Stimulation of Left Atrial Receptors on Tracheal Tension

During 10 observations in three animals, the effect of stimulation of left atrial receptors on tracheal tension was examined. Left atrial receptors were stimulated by applying weights to sutures attached to the pulmonary vein-atrial junctions by a pulley system. The average total weight attached to the three pulmonary vein-left atrial junctions was 276 ± 31 g. Heart rate increased from a control level of 130.8 ± 5.8 beat/min to 158.2 ± 7.5 beat/min ($p<0.05$) during this manoeuvre, while the corresponding mean aortic pressure was 133.6 ± 4.4 mm Hg and 132.2 ± 3.8 mm Hg respectively. Baseline tracheal tension was 87.3 ± 6.4 g. When left atrial receptors were stimulated, tracheal tension remained unchanged. Left atrial pressure was 7.1 ± 0.3 mm Hg during the control period and became 7.6 ± 0.5 mm Hg when the atrial receptors

were stimulated. This change was not statistically significant ($p>0.05$) (Table 14).

Protocol IVE. Effect of Partial Obstruction of the Tricuspid Valve on Tracheal Tension

In four dogs, 12 observations were made during partial obstruction of the tricuspid valve. It was not possible to increase right atrial pressure (a low pressure system) by an amount similar to that of the left atrial pressure, without impeding systemic venous return and disrupting the circulation excessively. Thus, the right atrial pressure was raised by an amount that caused a similar increase in heart rate as that observed when the left atrial pressure was raised by 10 mm Hg. Right atrial pressure was 5.1 ± 0.3 mm Hg during the control period and increased to 7.5 ± 0.4 mm Hg ($p<0.05$) during partial obstruction of the tricuspid valve. Heart rate increased from 171.0 ± 8.0 beat/min to 194.0 ± 5.0 ($p<0.05$) during the procedure while mean aortic pressure changed from 111.0 ± 5.0 mm Hg to 107.0 ± 7.0 mm Hg ($p>0.05$) (Table 15).

Baseline tracheal tension was 81.3 ± 8.9 g. During partial obstruction of the tricuspid valve and elevation of right atrial pressure, tracheal tension remained unchanged at 81.3 ± 8.9 g.

Protocol IVF. Effect of Stimulation of Right Atrial Receptors on Tracheal Tension

This protocol was similar to Protocol IVD and examined whether stimulation of the right atrial receptors without changing the right atrial pressure would affect the tracheal tension.

Under Protocol IVF, in five observations in five dogs, the superior vena cava right atrial junction was stretched to stimulate

the right atrial receptors situated at this junction. Heart rate increased from a baseline value of 157.2 ± 6.4 beat/min to 175.2 ± 6.1 beat/min ($p<0.05$) with stretching of the superior vena cava right atrial junction. Mean aortic pressure and mean right atrial pressure were 106.4 ± 5.1 mm Hg and 4.6 ± 0.5 mm Hg respectively. When right atrial receptors were stimulated, these pressures were 106.4 ± 5.1 mm Hg and 4.4 ± 0.3 mm Hg, respectively. No significant changes were noted in either of these pressures ($p>0.05$).

Baseline tracheal tension before stretching of the superior vena cava right atrial junction was 95.7 ± 4.3 g. During stretching of the superior vena cava right atrial junction, this remained unchanged at 95.7 ± 4.3 g (Table 16).

Protocol IVG. Effect of Sectioning the Superior Laryngeal Nerve on Tracheal Tension during Pulmonary Venous Congestion

In three dogs, following the completion of the first part of the observation in Protocol IVA, i.e., after showing that the tracheal segment increased its tension during elevation of left atrial pressure reversibly, the superior laryngeal nerves were exposed bilaterally and sectioned. Then after allowing 10 min for re-equilibration, control observations were made, to be followed by raising of left atrial pressure as in Protocol IVA.

Baseline tracheal tension was 91.2 ± 4.4 g and this remained unchanged when left atrial pressure was elevated from 6.8 ± 0.4 mm Hg to 16.3 ± 0.3 mm Hg. Heart rate during the control period was 143.0 ± 9.0 beat/min and when left atrial pressure was elevated, it became 185.0 ± 21.0 beat/min. Corresponding mean aortic pressures were 120.7 ± 4.2 mm Hg and 115.0 ± 5.2 mm Hg respectively.

The results of protocols IVA to IVF are summarized in Tables 10 to 16.

The increase in heart rate could be regarded as a baroreceptor mediated response to the sudden drop in blood pressure. However, some evidence indicates that other factors are involved. Firstly, in some of the experiments in protocol IV, while the mean arterial pressure was maintained at control levels during elevation of left (and right) atrial pressure, the heart rate still increased when either atrium was stretched. Secondly, in experiments in which there was stretching of either atrium without changes in systemic or intra-atrial pressures, the heart rate also increased. Thus, it may be concluded that the main factor in causing an increase in heart rate was due to stimulation of the atrial receptors. This conclusion is consistent with a number of previous studies reporting that stimulation of atrial receptors caused a reflex increase in heart rate (156,164,167,168).

In the present study, mean and peak airway pressures showed small consistent but statistically significant increases during pulmonary venous congestion. These changes were noted (see Fig. 24) almost immediately on increasing the left atrial pressure, before reflex contraction of the tracheal segment occurred, and returned to control levels almost immediately following reduction of the left atrial pressure to baseline levels. The changes were also noted, though to a much smaller degree, when the vagi were cooled to 8°C, when reflex contraction of the tracheal segment was abolished. The significance of these changes in causing stimulation of the pulmonary receptors and their contribution to the bronchospasm of cardiac asthma will be discussed later.

TABLE 10

Protocol IVA(i)

Effect of Pulmonary Venous Congestion on Tracheal Tension (37°C)

	CONTROL		PULMONARY VENOUS CONGESTION
Heart Rate (b/m)	139±10	p<0.001	181±6
Mean Arterial Pressure (mm Hg)	122±3	p>0.05	121±3
Mean Left Atrial Pressure (mm Hg)	4.6±0.6	p<0.001	15.3±0.6
Mean Airway Pressure (mm Hg)	2.23±0.19	p<0.05	2.55±0.17
Peak Airway Pressure (mm Hg)	5.29±0.19	p<0.05	5.79±0.18
TRACHEAL TENSION (g)	91.8±2.1	p<0.001	98.3±2.2

n = 53 in 17 animals

TABLE 11

Protocol IVA(ii)

Effect of Pulmonary Venous Congestion on Tracheal Tension during Vagal Cooling (8°C)

	CONTROL		PULMONARY VENOUS CONGESTION
Heart Rate (b/m)	162±11		168±6
Mean Arterial Pressure (mm Hg)	133±10		132±8
Mean Left Atrial Pressure (mm Hg)	5.3±0.4	$p<0.001$	15.1±0.7
Mean Airway Pressure (mm Hg)	2.30±0.28		2.44±0.28
Peak Airway Pressure (mm Hg)	5.22±0.29		5.44±0.28
TRACHEAL TENSION (g)	91.4±1.5		91.4±1.5

n = 5 in 5 animals

TABLE 12

Protocol IVB

Effect of Propranolol on Tracheal Tension

during Pulmonary Venous Congestion

	CONTROL		PULMONARY VENOUS CONGESTION
Heart Rate (b/m)	104±16		108±19
Mean Artérial Pressure (mm Hg)	142±12		141±12
Mean Left Atrial Pressure (mm Hg)	5.2±1.9	p<0.001	15.5±5.5
Mean Airway Pressure (mm Hg)	2.00±0.00		2.00±0.00
Peak Airway Pressure (mm Hg)	3.38±0.33		3.40±0.33
TRACHEAL TENSION (g)	101.3±4.1	p<0.05	109.0±6.6

n = 4 in 4 animals

TABLE 13

Protocol IVC

Effect of Atropine on Tracheal Tension

during Pulmonary Venous Congestion

	CONTROL		PULMONARY VENOUS CONGESTION
Heart Rate (b/m)	132±15	p<0.05	154±7
Mean Arterial Pressure (mm Hg)	135±10		143±11
Mean Left Atrial Pressure (mm Hg)	5.8±3.9	p<0.01	14.5±3.5
Mean Airway Pressure (mm Hg)	2.00±0.00		2.00±0.00
Peak Airway Pressure (mm Hg)	3.16±0.37		3.22±0.42
TRACHEAL TENSION (g)	97.7±3.9		97.7±3.9

n = 4 in 4 animals

TABLE 14

Protocol IVD

Effect of Stimulation of Left Atrial Receptors

on Tracheal Tension

	CONTROL		PULMONARY VENOUS CONGESTION
Heart Rate (b/m)	131±6	p<0.05	158±8
Mean Arterial Pressure (mm Hg)	134±4		132±4
Mean Left Atrial Pressure (mm Hg)	7.1±0.3		7.6±0.5
Mean Airway Pressure (mm Hg)	1.50±0.20		1.70±0.20
Peak Airway Pressure (mm Hg)	2.96±0.36		3.04±0.45
TRACHEAL TENSION (g)	87.3±6.4		87.3±6.4

n = 10 in 3 animals

TABLE 15

Protocol IVE

Effect of Partial Obstruction of Tricuspid Valve on Tracheal Tension

	CONTROL		PULMONARY VENOUS CONGESTION
Heart Rate (b/m)	171±8	p<0.05	194±5
Mean Arterial Pressure (mm Hg)	111±5		107±7
Mean Right Atrial Pressure (mm Hg)	5.0±0.3	p<0.05	7.3±0.5
Mean Airway Pressure (mm Hg)	1.90±0.18		2.00±0.13
Peak Airway Pressure (mm Hg)	3.12±0.40		3.14±0.38
TRACHEAL TENSION (g)	81.3±8.9		81.3±8.9

n = 12 in 4 animals

TABLE 16

Protocol IVF

Effect of Stimulation of Right Atrial Receptors on Tracheal Tension

	CONTROL		PULMONARY VENOUS CONGESTION
Heart Rate (b/m)	157±6	p<0.05	175±6
Mean Arterial Pressure (mm Hg)	106±5		104±5
Mean Right Atrial Pressure (mm Hg)	4.6±0.5		4.4±0.5
Mean Airway Pressure (mm Hg)	1.70±0.30		1.80±0.20
Peak Airway Pressure (mm Hg)	3.14±0.45		3.26±0.37
TRACHEAL TENSION (g)	95.7±4.3		95.7±4.3

n = 5 in 5 animals

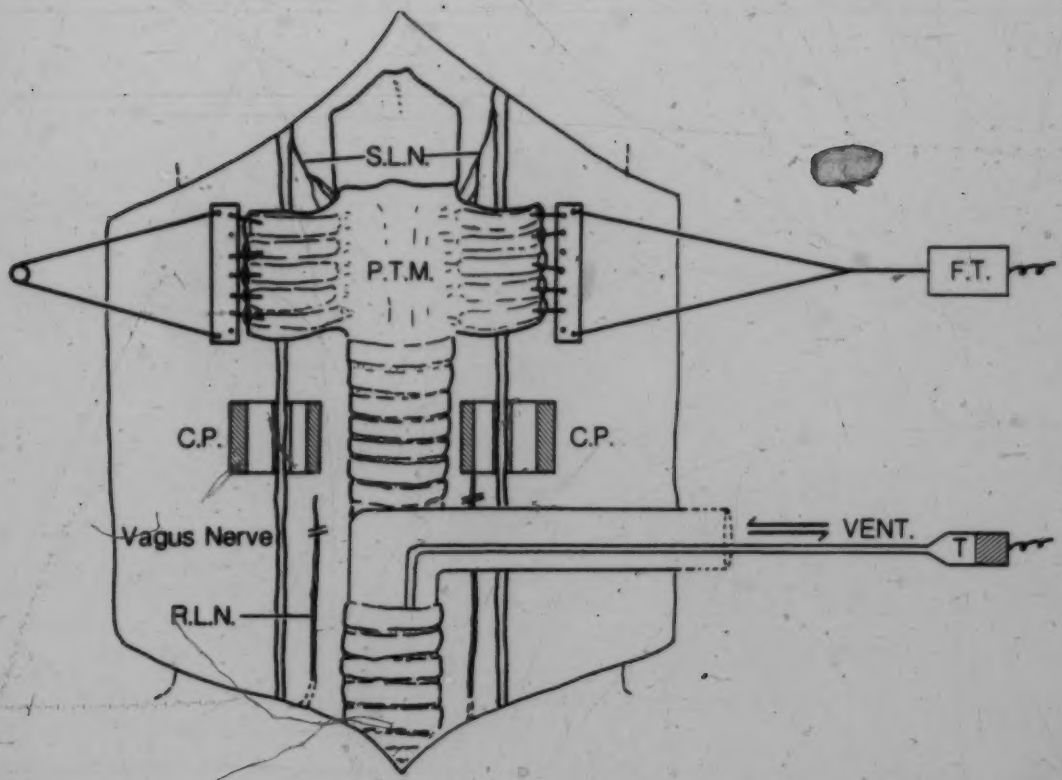

Fig. 20. Measurement of tracheal tension. A ventral midline incision was made to expose the trachea and vagus nerves in the neck. The dog was ventilated (VENT.) via a tracheostomy at the lower cervical trachea. Airway pressure at the level of the carina was monitored by a cannula placed in the tracheostomy tube and connected to transducer T. The vagus nerves were placed on cooling platforms (C.P.). The recurrent laryngeal nerves (R.L.N.) were cut while the superior laryngeal nerves (S.L.N.) supplying the upper tracheal segment were left intact. The upper trachea was incised ventrally and the cut edges incised around dorsally and retracted laterally to expose the posterior trachealis muscle (P.T.M.). Each cut segment was attached to a light plastic bar. One segment was attached to a fixed metal rod and the other to an isometric force transducer (F.T.) for measurement of tracheal tension.

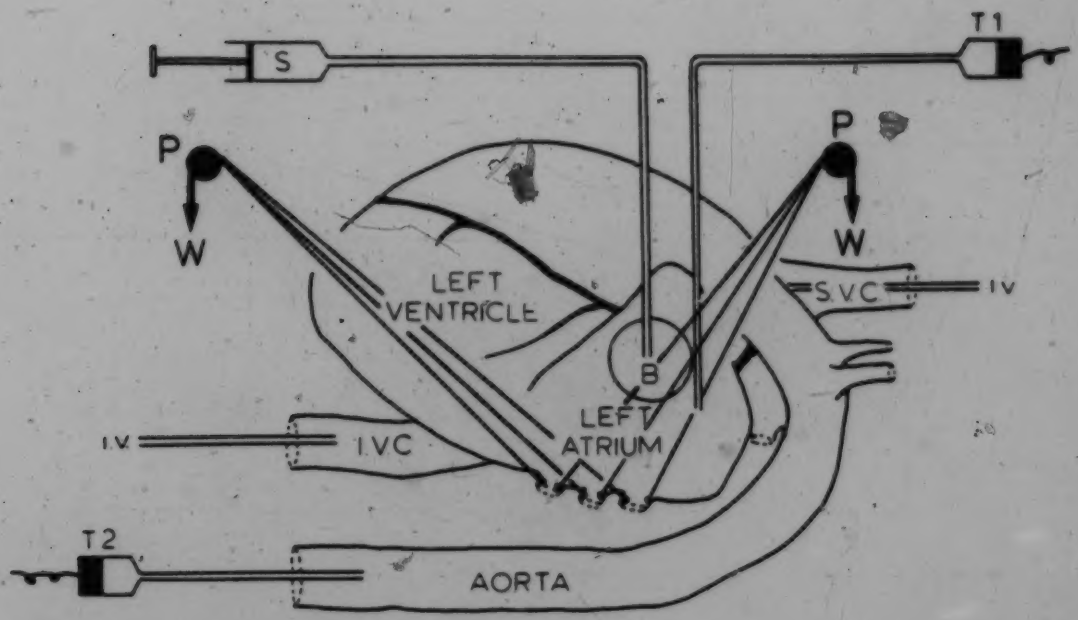

Fig. 21. Stimulation of left atrial receptors by stretching of the left pulmonary venous-atrial junctions. Through a left thoracotomy, the left side of the heart was exposed and the pericardium over the left atrium dorsal to the left phrenic nerve was incised longitudinally and then dorsally to each of the 3 pulmonary venous-atrial junctions. Two surgical silk ligatures were inserted into the vessel wall without puncturing through the vessel wall into the lumen. The ligatures were then directed ventrally, one in a cranial and the other in a caudal direction as shown. Weights (W) attached to these ligatures over a pulley system (P) caused stretching of the venous-atrial junctions and stimulation of the left atrial receptors situated at these junctions. Left atrial and intra-aortic pressures were monitored by cannulae connected to transducers T1 and T2 respectively.

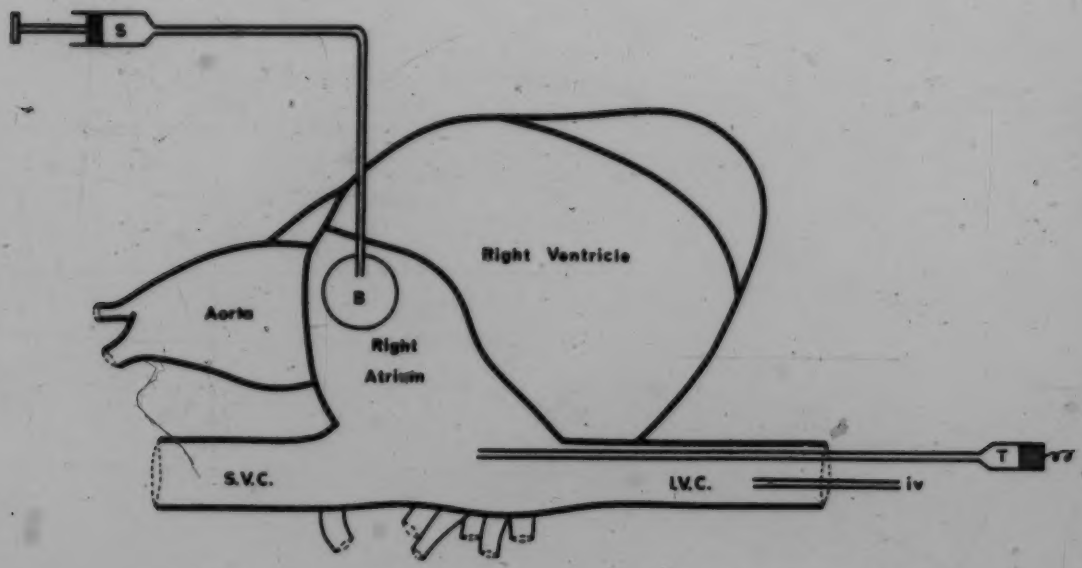

Fig. 22. Partial obstruction of tricuspid valve. Following exposure of the heart by a midline sternotomy, a balloon tipped catheter (B) was positioned in the right atrium via an incision in the right atrial appendage. A cannula connected to a pressure transducer (T) was positioned in the right atrium via the right femoral vein and inferior vena cava (I.V.C.). Distension of the balloon B with the syringe S caused partial obstruction of the tricuspid valve and elevation of right atrial pressure.

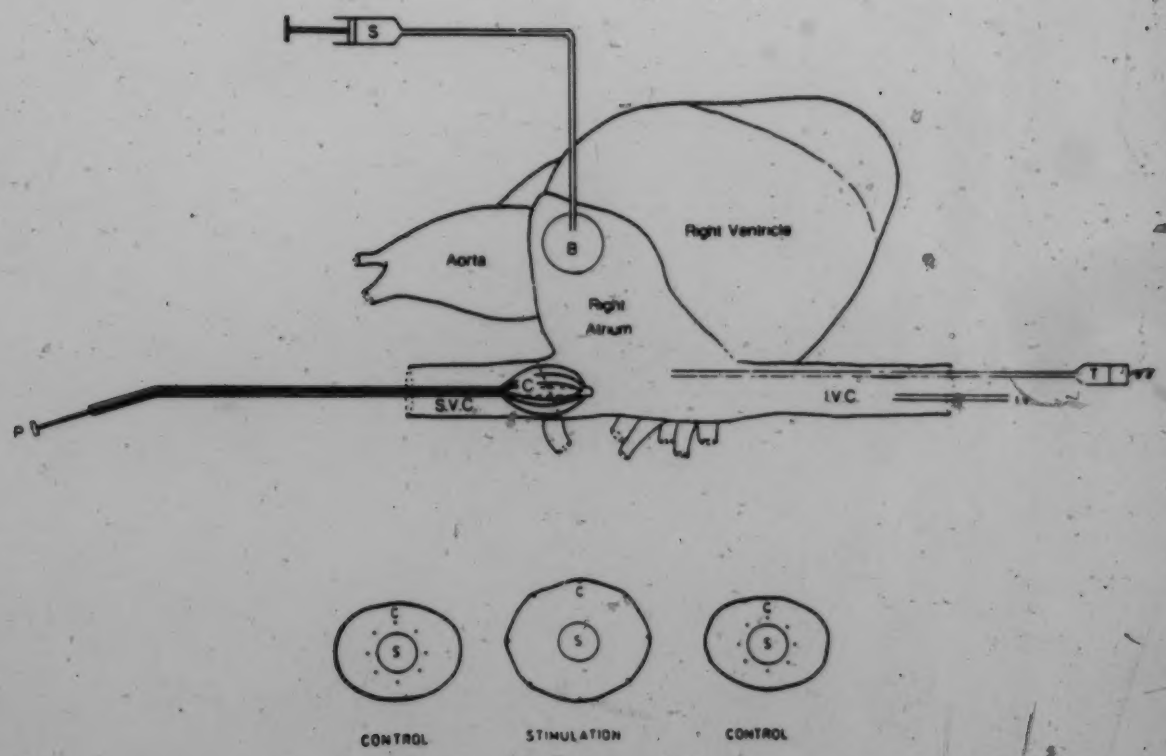

Fig. 23. Stretching of the superior vena caval-atrial junction. An expansile cannula was inserted via the right external jugular vein into the superior vena caval-venous junction and the position was confirmed by manual palpation of the heart through the sternotomy. Pushing piston (P) in the cannula caused expansion of the cage (C) attached to an inner metal tube S. The lower part of the diagram shows the effect of expansion of the cage on the junction during stimulation (centre figure) and the position of the cage during initial and final control periods (left and right figures respectively).

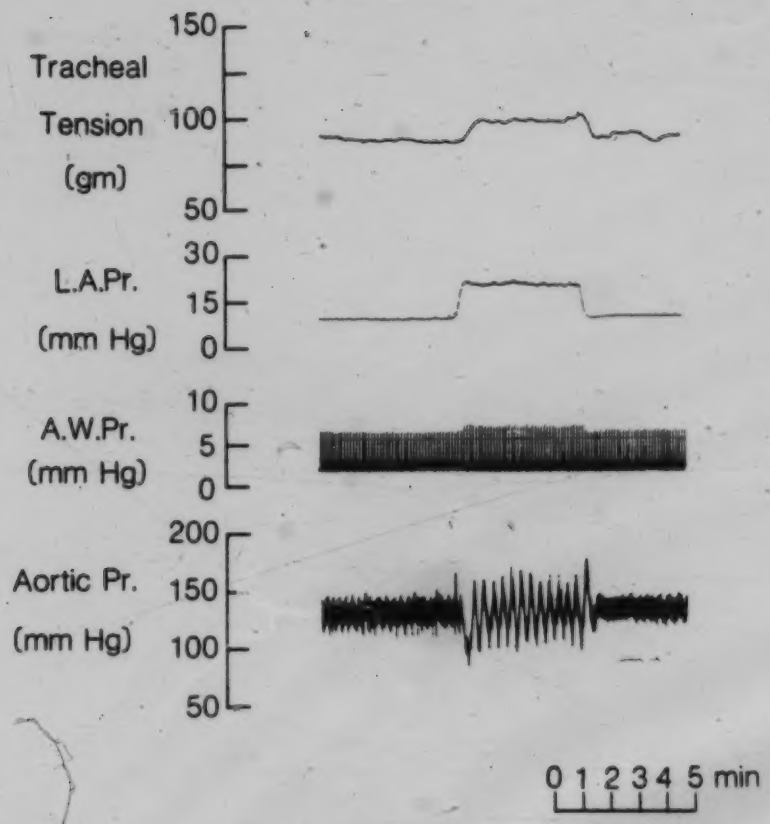

Fig. 24. An example of the effect of elevation of the left atrial pressure on tracheal tension. The tracings show, from above downwards: tracheal tension (gm), left atrial pressure (L.A.Pr., mm Hg), intra-tracheal airway pressure (A.W.Pr.) and aortic pressure (Pr.) (mm Hg). Tracheal tension increased from 83 gm to 98 gm after a delay of 30s following elevation of left atrial pressure from 10 mm Hg to 22 mm Hg. Tracheal tension then returned to basal control levels 30s after left atrial pressure returned to control. Note the small but obvious increase in peak airway pressure (A.W.Pr.) which occurred immediately following the increase in left atrial pressure and before tracheal contraction had fully developed. Mean aortic pressure did not change significantly but there were greater "swings" during elevation of left atrial pressure.

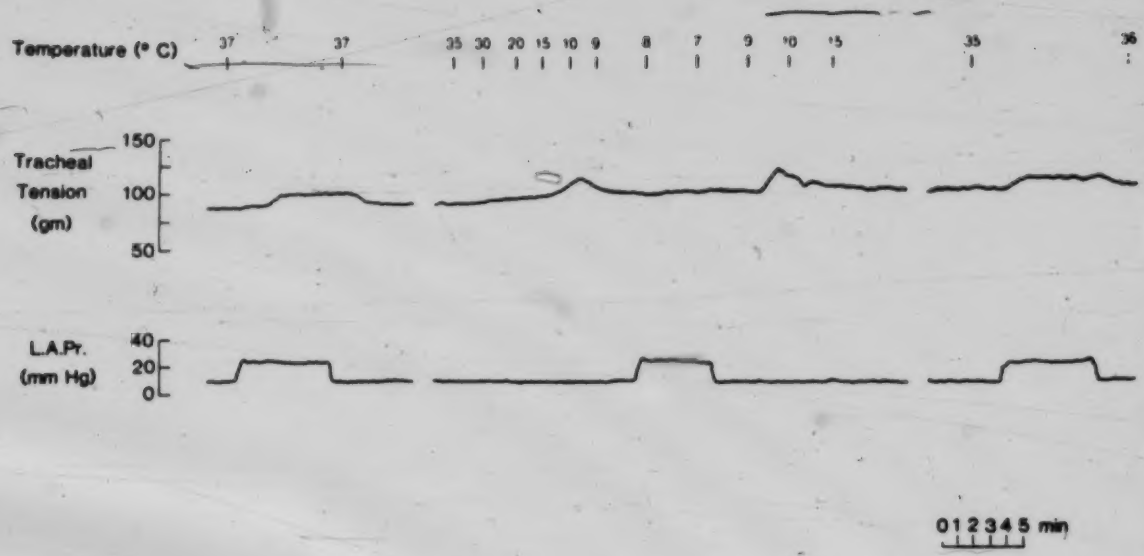

Fig. 25. An example of the effect of vagal cooling on the response of the tracheal tension to increases in left atrial pressure. The diagram shows from above downwards: temperature of the the vagus in the cooling platform (^{0}C) during gradual cooling and rewarming, tracheal tension (gm) and left atrial pressure (L.A.Pr., mm Hg). Tracheal tension increased in response to the increases in left atrial pressure at body temperture before vagal cooling and after rewarming. The response was abolished at vagal temperatures of 7-8^{0}C. Note the spontaneous contraction of the tracheal segment at temperatures between 9 to 15^{0}C.

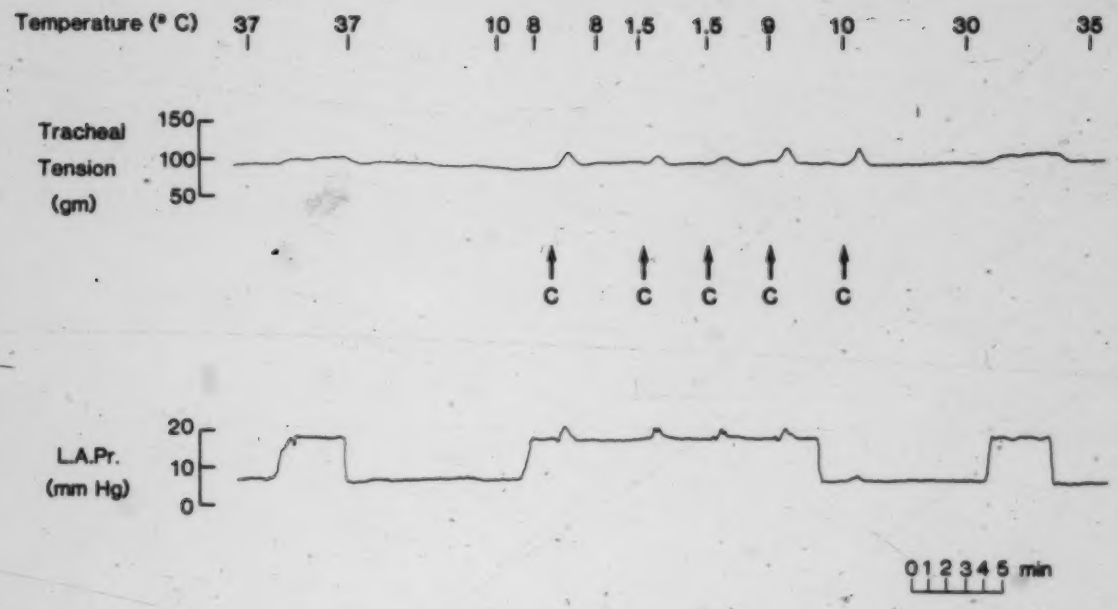

Fig. 26. An example of the effect of capsaicin on tracheal tension during vagal cooling with and without increases in left atrial pressure. The diagram shows from above downwards: vagal temperature (°C) during gradual cooling and rewarming, tracheal tension (gm) and left atrial pressure (L.A.Pr., mm Hg). Note that tracheal tension increased in response to the increases in left atrial pressure at body temperture before vagal cooling and after rewarming. The response was abolished at vagal temperatures of 7-8°C. At temperatures of 8°C and 1.5°C, tracheal tension increased transiently following capsaicin (C) administration into the right atrium. The contraction noted at 8°C was greater than that at 1.5°C.

CHAPTER 12

DISCUSSION

In the study on cardiac asthma, there is a need to investigate the link between the presence of pulmonary congestion leading to pulmonary interstitial oedema, i.e., a pathological state, and the clinical manifestation of respiratory wheeze. It is generally accepted that this link is "bronchospasm". The questions which should be posed are (a) does a nervous mediated bronchoconstriction contribute to the bronchospasm, (b) is the bronchospasm due to the local effects of the interstitial oedema on the airways and lungs?

A few studies have suggested that, at least in pulmonary oedema, a reflex bronchoconstriction does exist (5,6). However, these studies made use of indirect methods to detect changes in bronchial tone. They suggested that the afferent limb of the reflex was in the vagi, and did not identify specific receptor types which could be involved in the reflex. Very often, in these and other studies, pulmonary congestion and pulmonary oedema were studied together as a single entity.

As part of the study on cardiac asthma, the experiments described in this thesis were designed to answer some of the above questions. This part of the discussion will be concerned with the methods used in this study and their adequacy for answering the questions raised in the preceding paragraphs.

The results of the experiments are summarized here. Fourteen of 15 SAR, 11 RAR and 9 bronchial C-fibre receptors showed significant sustained increases in activity (+18%, +153% and +50% respectively,

$p<0.005$) when left atrial pressure was elevated by 9.4 $\pm$ 0.2 mm Hg. Six of nine pulmonary C-fibre receptors also increased their acivity but overall, the change in activity in this group was not significant. Also, all seven SAR, five RAR and five bronchial C-fibre receptors showed significant graded increases when left atrial pressure was increased in steps of 5 mm Hg each up to 15 mm Hg. Five pulmonary C-fibre receptors also showed graded, but not statistically significant, increases in activity. It was found that the RAR showed the greatest sustained increase in activity.

A further protocol showed that vagotomy and vagal cooling to 8°C, cranial to the site of recording the afferent activity, did not affect this response of the RAR to pulmonary venous congestion. Bilateral carotid occlusion similarly did not have any effect on RAR activity.

A significant 5-fold increase in lymph was collected from the lungs when left atrial pressure was elevated by 9.7 $\pm$ 2.1 mm Hg. Step-wise increases in left atrial pressure in steps of 5 mm Hg each up to 15 mm Hg caused small but significant decreases in dynamic compliance of the lungs when the left atrial pressure was > 10 mm Hg.

Tracheal tension increased reflexly by 6.5 $\pm$ 0.6 g from a baseline tension of 91.8 $\pm$ 2.1 g ($n = 53$ in 17 animals, $p<0.001$) when left atrial pressure was raised by 10.7 $\pm$ 0.2 mm Hg. This reflex was reversibly abolished by cooling the vagi to 8°C. The reflex was abolished also by vagotomy, administration of atropine and cutting the superior laryngeal nerves (motor supply). Propranolol or stimulation of atrial receptors without associated increases in left atrial pressure had no effect on the tracheal tension.

CRITIQUE OF METHODS

NATURE OF THE STIMULUS

Production of Pulmonary Venous Congestion

The method of inflating a balloon in the left atrium and partially obstructing the mitral valve was originally used to stimulate left atrial receptors in experiments with atrial receptor reflexes (158). Pulmonary venous congestion is produced when the elevated left atrial pressure produces back pressure into the pulmonary veins. It could be asked whether this method of production of pulmonary congestion is appropriate or natural. This is discussed below.

Guyton and Lindsey (7) employed a model similar to that used in the present study to examine changes in pulmonary fluid volumes. They observed that at normal plasma protein concentrations, pulmonary oedema occurred after 30 min, only when the left atrial pressure exceeded 25 mm Hg. Similarly, Jones et al, using a method used similar to that described in the present study observed that pulmonary oedema did not occur when left atrial pressure was elevated by 10 mm Hg over control levels for an hour or more (6).

Thus, it would appear that increasing the left atrial pressure by 10 mm Hg for up to 15 min or increasing the left atrial pressure by 15 mm Hg for 5 min would not be associated with the development of pulmonary oedema in the healthy animal. It was found in this study that producing pulmonary congestion by this method was easily and rapidly reversible. The left atrial pressure would return to control values rapidly, usually within a few seconds but always not longer than one minute following the deflation of the intra-atrial

balloon. If pulmonary oedema had occurred, it would be expected that the activity of the receptors would remain elevated after deflation of the intra-atrial balloon and would only gradually return to the control level as it would take time for the pulmonary oedema fluid to resolve (37).

Thus, it can be concluded that the method of producing pulmonary congestion in this way is indeed suitable for experiments of this type.

IDENTIFICATION OF PULMONARY RECEPTORS

Pulmonary receptor activity was recorded as single fibre action potentials from the cervical vagus nerve. A number of methods were used to identify these receptors.

Recording of Action Potentials

Recordings of nerve action potentials were carried out using established methods (156,234). It was possible to feed the signals into a discriminator and the action potentials could be counted electronically. There was no difference noted when simultaneous recordings of both the "raw" and " processed" signals were counted manually.

Measurement of Conduction Velocity

The method of measuring conduction velocity in this study has been established previously (156). The cathode of the stimulating electrode was always placed proximal to the recording electrode. In order to minimise the current spread during stimulation of the nerve, the vagus was grounded between the recording and stimulating electrodes at a point at least 2 to 3 cm from the recording electrode.

The mean strength of current used in this study to stimulate the myelinated fibres was above that reported by other investigators (134) despite the precautions taken. However, other than one unit, which required a strength of 80 V, the rest required much lower current strengths consistent with those reported previously (134). For the non-myelinated fibres, the current strength used for stimulation were similar to that used in earlier studies (156,237).

On the basis of measurement of conduction velocity, the fibre studied was differentiated into either myelinated or non-myelinated.

Other Criteria

The SAR were identified by their pattern of discharge with the phases of respiration, rapid conduction velocity, and low adaptation indices.

With regard to the RAR, as noted earlier in the literature review, there was no single universally accepted method of computing the adaptation index. After reviewing the methods used by other investigators (55,57,113,114), it was decided to define precisely the method of calculating the adaptation index used in this study in order to avoid ambiguity and also to avoid including receptors which have been classified as "intermediate" by Knowlton and Larrabee (55) and by Widdicombe (57).

The identification of C-fibre receptors by injection of foreign chemicals has been described previously by several investgators (39,58,59,60,131,134). In the dog, as the pulmonary C-fibre receptors are not usually stimulated by phenyldiguanide (60,131), it was possible to use capsaicin to identify pulmonary C-fibre receptors and phenyldiguanide to identify bronchial C-fibre receptors. A pulmonary

C-fibre receptor would respond within 3 seconds to capsaicin injected into the right atrium while a bronchial C-fibre receptor would respond to phenyldiguanide 7 to 13 seconds after injection of phenyldiguanide into the right atrium (39,131,134). During the experiments in the current study, it was noted that repeated injection of capsaicin and phenyldiguanide tended to cause instability in the animal's haemodynamics. Capsaicin caused part of the pulmonary chemoreflex, i.e., transient bradycardia associated with systemic hypotension and an increase in left atrial pressure. Phenyldiguanide produced a transient hypertension without an obvious change in heart rate. When repeated doses of both drugs were given, there was a tendency for arrhythmias to arise. Atrial fibrillation, frequent ventricular premature beats, idioventricular rhythm and sustained and non-sustained ventricular tachycardia and fibrillation had been encountered in this study.

Localisation of Receptors

The receptors can be localised to the lungs and airways in 2 ways. They can be located by introduction of a probe, e.g., guided by a bronchoscope, into the lumen of the airway and stimulating the mucosa while single fibre action potentials are being recorded. An alternative method, in the open chested animal, is to probe the airways externally with a glass rod or fingers to elicite a response.

In this study, the location of the receptors was established by discrete palpation of the mediastinum and the lungs. In the case of the SAR and RAR, the localisation was limited to a segment of the bronchus 1 cm in length. In the case of the pulmonary and bronchial C-fibre receptors, the process was continued until the receptor was

localised to a segment of parenchymal tissue of the lungs.

Thus, of the 70 receptors investigated under protocol I, 51 (73%) had been localised and all were found in the intra-thoracic airways and lungs. Localisation was not attempted in the other units to avoid collapsing the lungs mechanically, as it was felt that such disturbances could affect the behaviour of the receptors. However, the activity of those receptors in which localisation was not attempted was identical to those which had been localised. Thus, it would be reasonable to assume that these "un-localised" receptors would be in the similar sites in the lungs and airways as those which had been located. In any case, where a receptor showed activity different from the norm, it had to be located in the lungs or airways before being accepted.

MEASUREMENT OF TRACHEAL TENSION

In this protocol, a segment of the upper trachea with its motor nerve supply intact was used to study the response of the tracheo-motor tone during pulmonary venous congestion. During preparation for the experiment, it was important not to damage the superior laryngeal nerves supplying the motor innervation to the segment. The recurrent and para-recurrent laryngeal nerves were sectioned for several reasons.

By cutting these nerves, it was possible to study separately the efferent activity in the tracheal segment in response to the afferent input from the lower airways. Conduction in the cervical vagi caudal to the tracheal segment could be blocked effectively without interrupting the motor supply to this segment. Thus, it was possible, in this protocol, to selectively block conduction in the myelinated

afferent fibres at 7 to 8°C and demonstrate that the tracheal segment failed to contract in response to the stimulation from the pulmonary venous congestion. This effect was reversible on rewarming of the vagi. Also, when capsaicin, which stimulated C-fibre receptors as well as having a direct stimulating effect on the tracheal smooth muscle, was given when the vagi were at 8°C, the segment contracted consistently. This demonstrated that the segment was capable of contracting in response to stimulation of the C-fibre receptors which are known to cause bronchoconstriction by a reflex effect (39). When the vagi were at 1 to 2°C, most of the non-myelinated afferent fibres were also blocked (63,64). Thus, the response of the segment to injection of capsaicin into the right atrium was considerably attentuated when the vagi were at this temperature.

The recurrent laryngeal nerves were sectioned to avoid the influence of sensory inputs from the tracheal segment, arising from "irritation" due to blood, excessive handling, cooling or drying of the mucosal surface. Since the superior laryngeal nerves also supplied sensory innervation to the larynx and surrounding upper airway, care was taken not to disturb the larynx and the tracheal mucosal surface during recording. Also, the mucosal surface of the tracheal segment was kept reasonably moist and warm by dripping warm saline (at body temperature) onto it between recordings.

That the motor supply was from the superior laryngeal nerves and was parasympathetic in nature was demonstrated by the abolition of the tracheal segment response to pulmonary venous congestion after sectioning of the superior laryngeal nerves and the intravenous administration of atropine respectively.

The main question that would be asked when using this technique is whether the intra-thoracic and intra-pulmonary airways respond in a fashion similar to the tracheal segment when pulmonary venous congestion was produced. This has been discussed in pages 74-76 and the consensus appears to be that they probably do respond in the same way. It has been suggested that the posterior trachealis muscle does indeed bear strong similarities to the bronchial smooth muscles structurally, mechanically and pharmacologically (39,201). Also, studies of reflexes with serial tantalum bronchograms examining reflex contraction of the lower airways and reflex contraction of the tracheal segment in response to capsaicin were shown to have a similar time course (199,202).

COMMENTS ON RESULTS

The results obtained in protocol I indicate that, of the four types of pulmonary receptors, the RAR showed the greatest sustained increases in activity in response to pulmonary venous congestion. A finding that has not been previously described is the reflex contraction of an innervated tracheal segment in response to this pulmonary congestion. The discussion that follows relates these findings to earlier reports.

Effects on the Pulmonary Receptors

As noted in the literature review, previous studies on the effects of pulmonary congestion and oedema on some of the pulmonary receptors were performed in various ways. Their findings raise several issues. Specifically, it is found that the intensity of the stimulus applied, i.e., the increase in pulmonary venous pressure, was usually in excess of the changes observed in common physiological

or pathophysiological settings (91), and that the stimulus was usually applied for a relatively short period of time (usually 1-2 min) (91,120).

A recent study investigated the effects of pulmonary congestion and oedema on the pulmonary receptor activity in the dog. Pulmonary oedema was created by infusion of fluid into the animals and "congestion" was produced by removal of a portion of blood afterwards (37). It appears that this study examined the effects of gross pulmonary oedema (as shown by the histological studies) rather than lung congestion.

There are several aspects of the present study which make it novel. The first is that the degree of pulmonary venous congestion was relatively small (increments in left atrial pressure up to 15 mm Hg above the resting control level). There is evidence to indicate that alveolar oedema was not involved (this will be discussed later). Also, the increase in pressure was maintained for a period of 15 min. It is also emphasized that the findings of this study are derived from animals with an open chest, during intermittent positive pressure ventilation.

Effect on the SAR

One of the earliest studies on the effect of pulmonary venous congestion was that reported by Bulbring and Whitteridge (238). They observed that in preparations of the perfused cat lung, pulmonary venous congestion (up to 20 cm H_2O) was without any significant effect upon the activity of the vagal stretch receptors. Later, Marshall and Widdicombe (91), re-examined the problem in anaesthetized cats (closed chest) and found that increments of the

left atrial pressure of 20-40 cm H_2O produced a significant increase in activity in the stretch receptors. In these studies, the period of stimuli was less than 2 min. There are no other investigations of a comparable nature in the dog.

In the present study, a small but sustained increase in the discharge of these receptors during periods of elevated left atrial pressure (by 10 mm Hg) which lasted 15 min was observed. The average increase of the order of 18% was noticed in practically all the receptors examined. In addition, in the "graded" experiments over a range of pressures up to 15 mm Hg, a graded response was observed. This response was evident particularly when the left atrial pressure was increased by 10 to 15 mm Hg.

Effect on the RAR

The effect of pulmonary venous congestion on irritant receptor activity was examined by Sellick and Widdicombe (120) in anaesthetized cats. Again, the left atrial pressure was increased by inflating a balloon in the lumen of the left atrium but the actual pressures in the left atrium were not recorded. The period of stimulation was 1 to 2 min and the responses were not consistent in all fibres examined.

In the present study in the dog, it was found that an increase in left atrial pressure produced a large sustained increase in the discharge from the RAR. An average increase in activity of 153% was found when left atrial pressure was increased by 10 mm Hg. All receptors consistently showed an increase. During graded increases in left atrial pressure, the RAR showed a graded response in receptor activity. It is of interest to note that in these latter experiments,

a relatively small increase in left atrial pressure of 5 mm Hg was sufficient to produce a nearly 50% increase in activity of the receptors studied though this change did not achieve statistical significance (using ANOVA for multiple treatment testing). With further increases in left atrial pressure, stepwise increases in RAR activity was noted.

In a further protocol investigating the RAR activity following reversible (vagal cooling) and irreversible (vagotomy) vagal blockade, at a site cranial to the recording electrode, it was found that these manoeuvres had no effect on the responses of the RAR during pulmonary venous congestion. Further, bilateral carotid occlusion, which altered the stimulus to the carotid baroreceptors (as shown by the changes in heart rate noted) and thus influence the output of the sympathetic nervous system, did not alter the activity of the RAR.

Effects on C-fibre Receptors

There has been considerable debate on the effect of pulmonary congestion upon the discharge from the C-fibre receptors originating from the lungs. In the case of the pulmonary C-fibre (J) receptors, Anand and Paintal (151) have demonstrated, in the cat, that pulmonary congestion caused by augmenting the inflow of blood into one lung activated the receptors in that lung. This phenomenon was not a consistent finding in all the receptors examined. The bronchial C-fibre and pulmonary C-fibre receptors were examined in the dog by Coleridge and Coleridge (145). In general, the majority of the receptors examined were activated when the pressures were increased to levels exceeding 20 mm Hg. Having reviewed the available

information, Coleridge and Coleridge (39) concluded that the pulmonary C-fibre receptors were more consistent in their responses to the changes in left atrial pressure.

In the present study, it was found that in protocol IA, 6 out of 9 pulmonary C-fibre receptors responded to an increase in left atrial pressure of 10 mm Hg. During graded increases in left atrial pressures, pulmonary C-fibre receptors showed small stepwise but insignificant increases in activity.

In the case of the bronchial C-fibre receptors, Coleridge and Coleridge (145) showed that partial obstruction of the mitral valve activated these receptors, but the phenomenon could not be demonstrated in all the receptors examined. In the present study, the 9 bronchial C-fibre receptors showed average increases by 50% when left atrial pressure was elevated by 10 mm Hg. Graded increases in receptor activity was also noted when left atrial pressure was increased in a step-wise manner.

In general, the behaviour of the C-fibre receptors over a period of stimulation for 15 minutes was comparable to that described by Coleridge and Coleridge (145). There was no significant adaptation in the activity of these receptors to increases in left atrial pressure.

Time Course of Responses

The responses in receptor activity were obtained within 1 min of increasing left atrial pressure and returned to control levels within 1 min of deflation of the left atrial balloon. This response was maintained, with little fluctuation, especially in the case of SAR and RAR, during the period for which the left atrial pressure was raised (15 min in protocol IA and 5 min each, up to a total of 15 min

in protocol IB).

This is of interest in speculating on the nature of the stimulus on the receptors during elevation of the left atrial pressure. Guyton and Lindsey (7) have shown that left atrial pressures of less than 25 mm Hg, a pressure higher than encountered in the present study, did not produce pulmonary oedema. They also showed that, when the left atrial pressure was greater than 25 mm Hg, pulmonary oedema occurred only after 30 min in animals with normal plasma protein concentrations. Similarly, Jones et al, using a dog model (closed chest) and a balloon in the left atrium to cause pulmonary venous congestion (pulmonary capillary wedge pressure up to 15 mm Hg) showed that pulmonary oedema did not occur even after one hour (6).

Thus, it would seem unlikely that oedema of the lung, i.e., alveolar oedema, was the cause in the increase in activity of the receptors observed in the present study. The nature of the stimulus on the receptors will be discussed later in this section.

EFFECTS ON TRACHEAL TONE

In this study, contraction of an upper innervated tracheal segment in response to a moderate degree of pulmonary congestion was demonstrated in the anaesthetized, open chest and artificially ventilated dog. The magnitude of this increase was 6.8 to 7.6% that of the resting baseline tracheal tension. That this response may be reflex in nature was suggested by the finding that it was rapidly reversible and in phase with the production and reduction of pulmonary venous congestion. Secondly, and more importantly, this response was reversibly abolished by cooling the vagi to 8°C.

SPECULATION ON MECHANISMS

Speculation on the Physiology of Stimulation of the Pulmonary Receptors, particularly the RAR, during Pulmonary Venous Congestion

Pulmonary venous congestion caused by partial obstruction of the mitral valve has been shown to increase the activity in SAR, RAR and bronchial C-fibre receptors in this study. In view of the nature of the stimulus used, it is of interest to speculate upon the nature of the change tranduced by these receptors.

1. Effect of a Change in Bronchomotor Tone

Since the SAR and RAR are located in the large airways (both extra- and intra-pulmonary) (97,107), it is possible that the changes observed in the activity in these receptors were mediated by a concurrent change in tone of the smooth muscle of the airways. In the dog, partial obstruction of the mitral valve produces a change in total pulmonary airway resistance. Hogg et al (239) and Jones et al (6), showed that this increase in resistance resided primarily in the smaller airways. However, the findings in protocol IV of this study, have shown that increments in the left atrial pressure of 10 mm Hg caused a reflex increase in tone in the trachea at a site immediately below the larynx. Thus, secondary influences of changes in bronchomotor tone upon receptor activity should be considered.

One approach to the problem is to examine the influence of agents which alter bronchomotor tone by a direct effect upon the smooth muscle of the airways. In the dog, acetylcholine increases bronchial tone by such a direct effect (105). The studies of Davenport et al (81) suggested that, in the dog, the changes in bronchomotor tone

necessary to bring about increases in the activity of SAR, resulted in increments in peak airway pressure in excess of 10 cm H_2O (7.4 mm Hg). The effect of changes in bronchomotor tone upon the activity of RAR in the dog were examined by Vidruk et al (122). They found that the activity of the RAR was almost unchanged even after the peak airway pressure had increased by more than 20 cm H_2O (14.8 mm Hg) following the injection of acetylcholine. There are no comparable studies on the bronchial C-fibre receptors in the dog. The changes in peak airway pressure which resulted from the stimulus employed in the present study were very small in comparison to those observed in the above investigations. Thus, a change in bronchomotor tone, per se, is unlikely to have produced the findings of the present investigation.

Further, bilateral carotid occlusion, a procedure which produces an enhancement in sympathetic activity, failed to influence both the discharge of the RAR in the control state and the effect of mitral valve obstruction on it.

2. Effect of a Change in Compliance of the Lung

In the present study, both the mean airway pressure and the peak airway pressure, as measured in the carina, increased when the left atrial pressure was raised. Since these changes in airway pressures persisted after sectioning the vagi (+0.35 mm Hg before and +0.23 mm Hg after vagotomy), it is likely that they were secondary to a change in the dynamic compliance when left atrial pressure was elevated by 10 mm Hg or more. It is not possible to establish with any certainty, the influence of changes in dynamic compliance signified by such small changes in airway pressures (+0.16 mm Hg for mean, +0.43 mm Hg for peak pressure - protocol IA) upon the activity of the pulmonary

receptors. In the cat, it was found that injection of micro-emboli into the pulmonary arteries increased the peak intra-tracheal pressure and the activities on RAR, SAR and pulmonary C-fibre receptors (92). Since the increase in peak intra-tracheal pressure persisted even after vagotomy, Armstrong et al (92), attributed it to a decrease in dynamic compliance of the lung. In their study, it was found that the activity of these receptors was not elevated in a persistent manner, until the peak airway pressure increased by more than 1 cm H_2O (0.74 mm Hg). If these findings could be extrapolated to the dog, a reduction in compliance, as signified by the magnitude of the changes in airway pressure and dynamic compliance observed in the present study, is unlikely to influence the activity of the receptors.

However, recent studies by Jonzon et al (240) in dogs, and by Yu et al (241) in cats and rabbits have shown that RAR were sensitive to changes in dynamic compliance and the authors speculated that the role of the RAR might be to help to maintain lung tidal volume as the lungs became stiffer. At first sight, this interpretation of results appears different from what is implied in this study, i.e., the mechanism of stimulation of the RAR. However, the extent of changes in dynamic compliance were larger in these studies than noted here. Also, it may be speculated that such changes in airway dynamic may in fact produce distortions in the submucosal interstitium and activate the RAR located there, by a process possibly involving local shifts in fluid fluxes.

3. Pulmonary Venous Congestion and Oedema

This stimulus of partial obstruction of the mitral valve

interferes with venous return from the lung, increases capillary filtration in the lung and eventually enhances the production of lymph. The occurrence of pulmonary oedema in this model has been investigated extensively by Guyton and Lindsey (7) and Jones et al (6). As referred to earlier, Guyton and Lindsey (7), showed that pulmonary oedema might occur after 30 min when the left atrial pressure was elevated to exceed 25 mm Hg in dogs with normal plasma proteins. Jones et al (6), using two separate methods to detect changes in lung water, demonstrated that no increase in lung water occurred when the pulmonary capillary wedge (left atrial) pressure was increased up to 15 mm Hg for one hour. Extrapolating from their findings, it could be suggested (within the limits of their methodology), that there is no significant oedema following exposure to an increase in left atrial pressure of 10 mm Hg for a period of 15 min. Further, if the changes observed in the present study were the consequence of interstitial oedema, it is likely that the effect upon receptor activity would be a progressive rather than an abrupt change when a constant stimulus was applied as in protocol IA.

4. Effect of the Bronchial Circulation

Apart from the issue of pulmonary oedema, it is conceivable that obstruction of the venous return from the lung could have a bearing upon the activity in the receptors. In addition to obstruction to flow from the lung parenchyma, it is likely that the venous return from the bronchial mucosa is interfered with. The proximal and distal bronchial mucosa derives its blood supply from the bronchial circulation but the venous return occurs via the pulmonary veins (20,134). Within the bronchi, the veins are located transversely and

obstruction, to flow in them could engorge the mucosal cell lining which contain the majority of the RAR (20,69,106,110,112). Further, elevation of pressure in these veins could lead to increases in local interstial fluid which, in turn, could result in changes in the matrix material in the interstitium. Such changes in the matrix could cause distortion in the architecture surrounding the RAR nerve endings and activate them (see chapt. 13, pg.197-206).

Indeed, Paintal suggested such a mechanism for activating the pulmonary C-fibre (J) receptors in the juxta-capillary and alveolar areas. He postulated that these receptors are found in the interstitial space surrounded by collagen fibres, which, in the presence of increased interstitial fluid during pulmonary oedema, would swell, become distorted and stimulate the receptor endings (140). Perhaps such a process could well be applied to the RAR endings found in the bronchial submucosal interstitium.

Such speculation is supported by the time course of the "on" and "off" effects of the stimulus on the RAR, with both effects being observed within 1 min of the change (242).

Speculation on the Mechanism of the Reflex Tracheal Contraction

It has been stated earlier that the response of the upper tracheal segment to the elevation of left atrial pressure was a reflex in nature.

Afferent Limb of Reflex

The reflex contraction was abolished on vagal cooling to 8°C, a temperature at which conduction in the myelinated nerve fibres in the vagi was preferentially blocked. Among the afferents discharging into

myelinated nerve fibres likely to play a role in this reflex were those from the pulmonary receptors (SAR and RAR), and from the atrial receptors. All these receptors would be stimulated when a balloon was inflated in the left atrium producing elevation of left atrial pressure (158).

Stimulation of the SAR have been shown to be associated with bronchodilatation (214), while stimulation of the RAR resulted in bronchoconstriction (100,108).

With the vagi cooled to 8°C, non-myelinated nerve fibres are known to be preferentially spared from blockade (63,64). This was demonstrated in this study by the administration of capsaicin into the right atrium. Capsaicin stimulated receptors discharging into non-myelinated fibres. At 8°C and following the injection of capsaicin, there was indeed a transient contraction of the tracheal segment in phase with the onset and duration of the haemodynamic effects of capsaicin, i.e., bradycardia and hypotension (39). The magnitude of the tracheal contraction was found to be the same both in the presence and absence of pulmonary venous congestion. This occurred in the absence of the reflex contraction in response to pulmonary congestion. At 1°C, when conduction in most of the non-myelinated fibres were also blocked, the magnitude of the tracheal contraction was significantly reduced. Thus, the findings suggest that the RAR most likely form the afferent link of this reflex.

Stimulation of the left atrial receptors alone without an associated increase in left atrial pressure was not accompanied by reflex tracheal contraction. The technique described in this study

has been used previously to study cardiovascular reflexes, in particular the response of the intrinsic heart rate to stretch of the pulmonary vein-left atrial junctions, in which a large number of atrial receptors are located (156,166,167,168). Stimulation of the pulmonary vein-left atrial junctions caused a reflex increase in heart rate, a result of stimulation of the atrial receptors. This increase in heart rate was shown to be a reflex by its abolition due to intravenous propranolol, which blocked the efferent chronotropic effect of sympathetic nerves on the SA-node of the heart. Stimulation of the atrial receptors in this way failed to bring about a reflex tracheal contraction.

The right atrial receptors were stimulated in two ways. Partial obstruction of the tricuspid valve caused stretching of the right atrial wall and stimulation of the atrial receptors, as indicated by a reflex increase in heart rate. In this study, care was taken to avoid activation of the baroreceptors. Stimulation of baroreceptors could lead to a reflex increase in heart rate as well have a reflex effect on the tracheal motor tone (223). Sudden, excessive reduction in aortic pressure was avoided by inflating a balloon in the descending aorta to counteract the tendency for the aortic pressure to fall when the tricuspid valve was partially obstructed. This failed to produce a reflex tracheal contraction.

The right atrial receptors were also stimulated by stretching of the superior vena cava right atrial junction by an expansile cannula. This device has been shown previously to cause a reflex increase in heart rate by stimulation of the right atrial receptors and not by a direct effect on the SA-node (168). This stimulation again failed to elicite reflex tracheal contraction. Thus, the evidence found so far

suggests that the atrial receptors have little or no role in the production of the reflex tracheal contraction in response to pulmonary congestion (243).

Hypercapnoea and hypoxia have potent influences on the tracheal tone (219). In this study, care was taken to avoid the influence of hypercapnoea and/or hypoxia by ensuring adequate ventilation and maintaining the pCO_2 and pO_2 in the normal range. Acidosis, which has a bronchodilating effect (221) and hypertonic solutions which may have a bronchoconstricting effect (224) were avoided by giving bicarbonate solution and other fluids only between observations. Similarly, the influence of aortic and carotid baroreceptors (223) was attenuated by artificially maintaining mean aortic pressure as close to control as possible during production of pulmonary congestion.

Efferent Limb of the Reflex

The motor innervation of the upper tracheal segment is by the recurrent laryngeal, para-recurrent laryngeal and superior laryngeal nerves (39,200). Prior to the study of the tracheal contraction in each experiment, both the recurrent and para-recurrent laryngeal nerves were cut. This was done so that the afferent and efferent links of the reflex could be studied separately, i.e., with the afferent limb arising from the lungs and the efferent limb passing through the superior laryngeal nerve. In this study, cooling of the vagi to block conduction in the afferent nerve fibres, caudal to the site from which tracheal tension was measured, was possible without affecting the efferent pathway.

The efferent pathway of this reflex appears to be mediated by cholinergic fibres as the reflex was abolished by the administration

of atropine. That the efferent fibres were in the superior laryngeal nerves was evident by the finding that interruption of the latter abolished the reflex.

In the preparation used in this study, the sympathetic nervous system appears to play a secondary role. If a significant degree of sympathetic mediated relaxation of the tracheal smooth muscle existed, a reflex contraction might be expected following beta-blockade (227,228). In this study, the resting tracheal tension did not change following the administration of propranolol.

The possibility that the observed tracheal contraction was a direct result of the stimulus applied in the lungs and airways has to be considered. One way this could occur is the development of pulmonary interstitial oedema extending into the upper trachea. A number of studies have shown, however, that when left atrial pressure and pulmonary venous pressure was raised to the level as in this study, interstitial oedema was not significant in the main bronchi (6). Further, the time course and the reversibility of the contraction and relaxation of the tracheal segment closely in phase with the changes in the left atrial pressure would be against such a direct effect. If interstitial oedema was the cause of the increased tracheal tension, one would expect an accumulative effect in the tracheal tension in the same animal when the left atrial pressure was elevated and reduced repeatedly. This was not noted in this study. Finally, abolition of the reflex by atropine and by the interruption of the superior laryngeal nerves would not support the hypothesis that the tracheal contraction was due to a direct effect of the oedema (244). It has been suggested that bronchial and peribronchial oedema do not appear to narrow directly the bronchial lumen (5).

CHAPTER 13

SPECULATION ON AN ALTERNATIVE HYPOTHESIS FOR THE EXCITATION OF OF THE RAR

Forty years after their identification, the natural stimulus for the excitation of the RAR is still unresolved. Enhancement of pulmonary extravascular fluid by various stimuli as found in this study, excites these receptors, suggesting that their natural stimulus may be a function of the fluid fluxes in the extravascular compartment.

The sensory receptors of the airways discharging into vagal afferents have been extensively investigated in experimental animals and several reviews of their properties have been published (59,84,140). It is now accepted that there are four principal types of vagal sensory receptors in the airways of cat and dog. These are: (i) SAR, (ii) RAR, (iii) bronchial C-fibre receptors and (iv) pulmonary C-fibre (J) receptors (140). Of these, the former two discharge into medullated fibres and the latter two into non-medullated fibres (84). The natural stimulus for the excitation of the SAR is either an increase in lung volume (54) or a change in airway pressure associated with an increase in lung volume (41,54,66). The natural stimulus for the stimulation of J receptors is considered to be an increase in pulmonary interstitial fluid volume (140). However, there are no firm opinions regarding the natural stimuli for the excitation of RAR and the bronchial C-fibre receptors. A hypothesis is presented here relating to the natural stimulus for the excitation of the RAR in the dog. This hypothesis is based upon some observations made in this laboratory on the

electrophysiology of the RAR (242) and the recent view expressed by Grega (22) on the gap junctions in endothelial cells of venules.

The RAR were identified as a separate group by Knowlton and Larrabee in 1946 (55). They were so named since they adapted rapidly to a sustained inflation of the lungs (55). Subsequent investigations revealed that they responded to a variety of chemicals such as SO_2, ammonia vapour (57), cigarette smoke and carbon dust (103). Since the receptors were excited by these "irritant chemicals", it was suggested that their natural stimulus was a non-specific irritation of the airways (59). Hence the bronchoconstriction that followed the inhalation of these chemicals was considered to be due at least in part to stimulation of the RAR (103). However, as pointed out in a recent review, none of these stimuli were likely to produce a direct mechanical distortion of the tissues at the receptor site (41). Also, rapid adaptation to a sustained inflation of the lungs is of no physiological importance since the animals do not hold their breath (140). Therefore, the identity of the natural stimulus for these receptors should be sought elsewhere.

In a study on anaphylactic shock, the RAR activity has been reported to be markedly increased (103). This effect was thought to be secondary to the contraction of the airway smooth muscle caused by histamine liberated during the development of the anaphylactic shock (103). Since an intravenous injection of histamine stimulated these receptors, they were implicated in the airway responses of asthma (126). Nevertheless, the issue of whether histamine acts directly on the receptors (122) or indirectly through changes in smooth muscle tone (105) remains to be resolved (84).

Background

During a brief period (1 to 2 min) of pulmonary congestion produced by elevation of left atrial pressure, the activity of the RAR increased before any change in the lung mechanics was detected (120). Similarly, following the release of pulmonary congestion by lowering the left atrial pressure, the activity promptly returned back to control, even though occasionally, the lung mechanics did not do so (120).

Hypothesis

During pulmonary congestion produced by elevation of left atrial pressure, there would be an increase in pulmonary venous pressure, which would be reflected upon the bronchial veins since the latter drain into the pulmonary veins (20). One consequence of this rise in pressure would be an increase in permeability to fluid of the bronchial venules resulting in an increase in extravascular volume. Based upon physiological and pharmacological studies, the hypothesis that the natural stimulus to these receptors would be a function of the extravascular fluid volume is presented here.

To test this hypothesis, two series of experiments were performed in which the responses of RAR were examined: (a) pulmonary congestion was produced for periods up to 15 min by partially obstructing the mitral valve (protocol I in this thesis) (242) and (b) in a further protocol pulmonary lymphatics were obstructed by increasing the afterload in the central veins into which they drained (124,245).

(a) In anaesthetised thoracotomised dogs, it is observed that elevation of left atrial pressure by 5 mm Hg consistently stimulated the RAR. A typical example is given in Fig. 27. The increase in

activity persisted throughout the period of partial mitral valve obstruction, showed no evidence of adaptation and returned to the control state immediately after lowering of the left atrial pressure (124,242,245)

(b) An isolated venous pouch of the right external jugular vein in situ near the points of entry of the pulmonary lymphatics was constructed (18). Details of the contruction of this pouch were given in protocol II. By pressurising this pouch, it was possible to obstruct the flow of pulmonary lymph in a reversible manner. During this period also, the receptor activity increased. Once again, there was no evidence of adaptation (124,245). A typical example is given in Fig. 28.

In both these conditions, there would be an expansion of extravascular fluid. Since the receptor activity increased without any significant change in the lung mechanics, it is likely that the primary stimulus for their excitation must be a derivative of fluid flux into the extravascular space.

Pharmacological studies

Further evidence in support of this hypothesis was obtained by examining the effects of pharmacological interventions on the receptor activity. Vasoactive substances like histamine and bradykinin produce bronchial oedema by increasing the permeability in the bronchial venules (20). With regard to the action of histamine on capillary endothelia, it has been shown that it widens the gaps between the endothelial cells and this widening increases the capillary permeability (22). It is hypothesised that if the natural stimulus for the RAR is a change dependent upon fluid fluxes in the

extravascular space, it would be possible to summate the responses by two stimuli which act through the same mechanism. For this experiment, a dose of histamine (0.25 µg/kg) was injected into the right atrium in the control phase. This dose of histamine neither stimulated the RAR nor produced any change in upper airway smooth muscle activity. However when this dose of histamine was reinjected during pulmonary lymphatic obstruction, it produced a marked excitation (246). A typical example is given in Fig.29.

Summary

Based on the evidence presented above, it is suggested that the natural stimulus for the RAR is a function of the fluid fluxes in the pulmonary extravascular space. In order to function in this manner, the RAR must be located in a site where changes in extravascular fluid could be detected (Fig. 30). Further confirmation of this hypothesis demands careful histological studies with sophisticated techniques of three dimensional reconstruction.

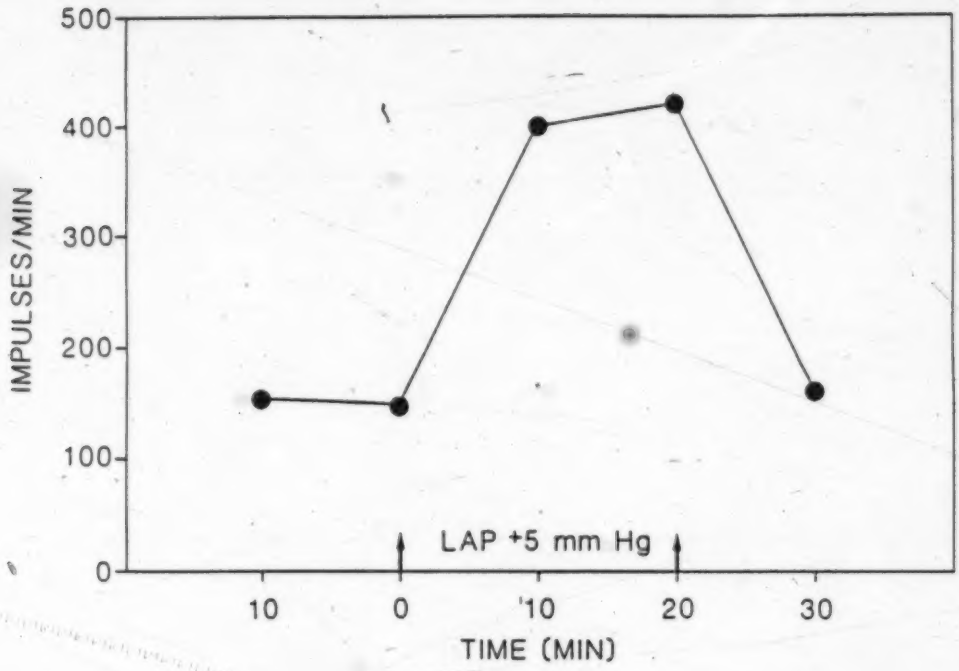

Fig. 27. Pulmonary congestion and RAR afferent activity. The neural activity was recorded for a control period of 10 min. Pulmonary congestion was produced between the arrow markers for 20 min by partially obstructing the mitral valve. During this manoeuvre, the left atrial pressure (LAP) increased by 5 mm Hg. Note the increase in neural activity and the lack of adaptation in the neural response in this period. On relieving the pulmonary congestion, the neural activity returned back to control.

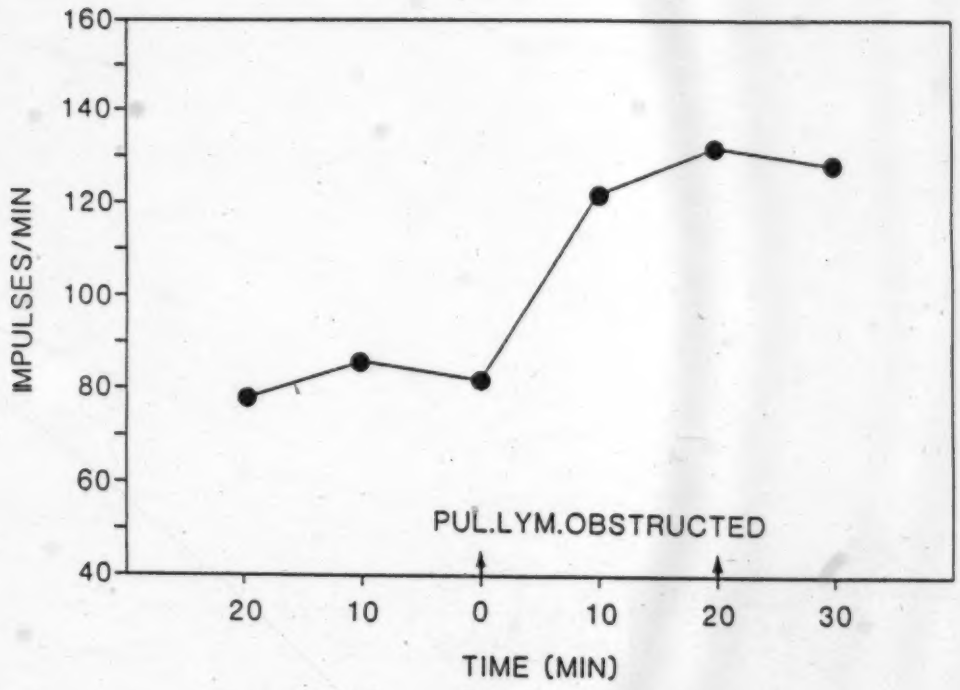

Fig. 28. Pulmonary lymphatic obstruction and RAR afferent activity. The neural activity was recorded for a control period of 20 min. Pulmonary lymphatic obstruction was produced between the arrow markers for a period of 20 min by increasing the afterload in the right external jugular vein. Note the increase in neural activity and the lack of adaptation in the neural response in this period.

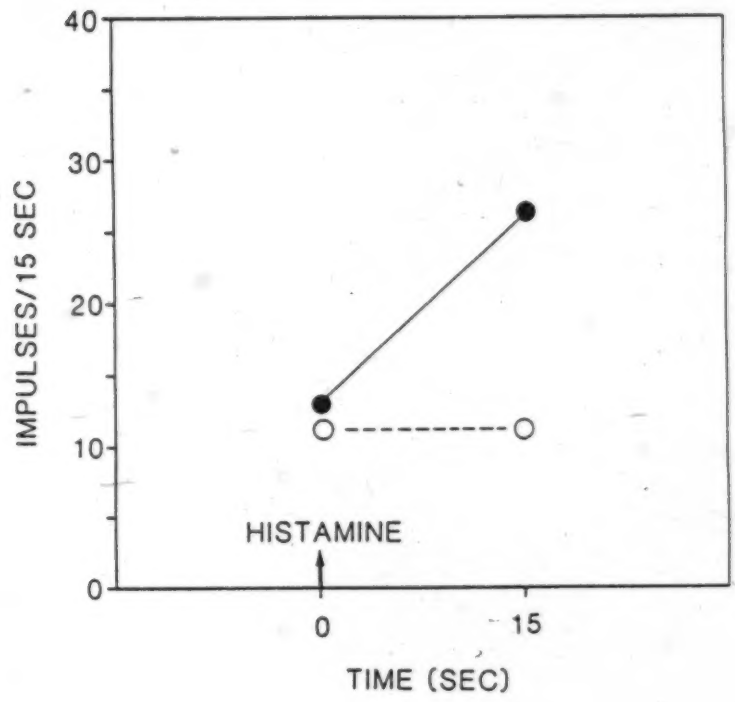

Fig. 29. Histamine and RAR afferent activity. Histamine (0.25 µg /kg) was injected into the right atrium at 0 time. In the control period, it produced no response in the neural activity (O---O). After pulmonary lymphatic obstruction was produced for 5 min, the same dose of histamine produced a marked increase in neural activity (●—●).

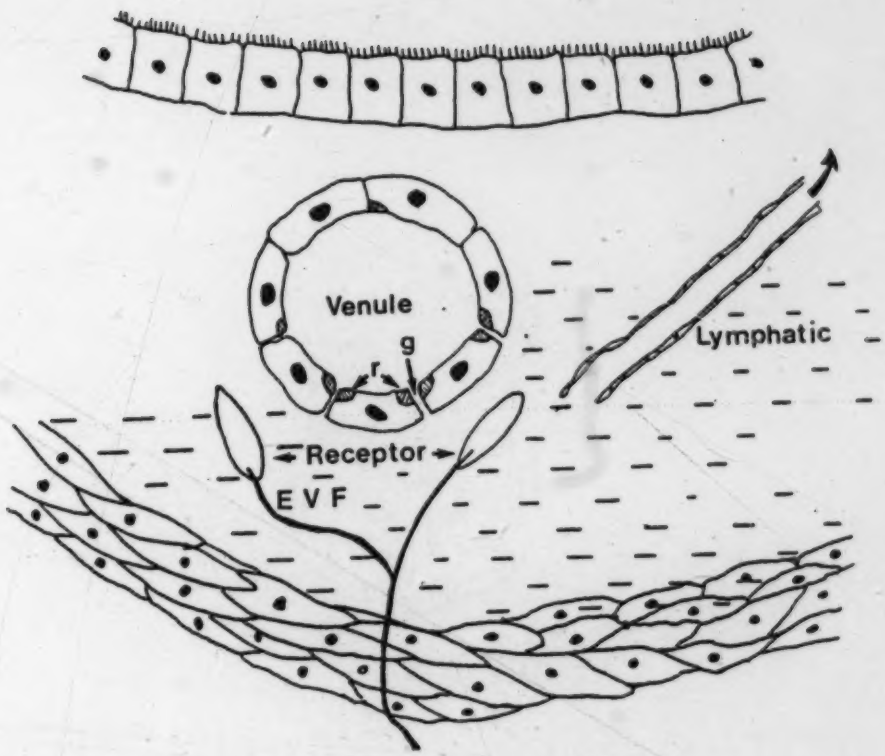

Fig. 30. Hypothetical location and the natural stimulus for the RAR activity. The receptor is located in the extravascular space surrounded by extravascular fluid (EVF). During pulmonary congestion, there would be an increase in the permeability of the bronchial venules which would increase the EVF volume. During pulmonary lymphatic obstruction, there would not be adequate draining of the EVF. Vasoactive substances like histamine would act upon the pharmacological receptors (r) on the endothelial cells of the venules, widen the gap (g) between them and increase their permeability. Consequently there would be an increase in EVF volume. An increase in EVF volume is transduced by the receptor.

CHAPTER 14

IMPLICATIONS FOR CARDIAC ASTHMA AND ITS MANAGEMENT

Speculation on a Mechanism in the Development of Respiratory Wheeze during Cardiac Asthma

Based on the results of the present study it is proposed that one of the mechanisms leading to the development of the respiratory wheeze during cardiac asthma may be as follows. Pulmonary congestion causes changes in fluid fluxes in the extra-vascular interstitial spaces of the lungs and airways in which the pulmonary receptors are located. Of particular interest is the bronchial circulation. Pulmonary congestion can readily cause interstitial oedema in the bronchial submucosa and engorgement of the mucosal cells. The RAR found in the bronchial submucosal areas may be activated by the rapid changes in fluid fluxes in this environment. The stimulated RAR may then form the afferent limb of the reflex which results in an increase in tone of the airways leading to narrowing of the airway lumen and respiratory wheeze.

Besides bearing similarity in the clinical features, the findings in this study suggest that both forms of asthma - bronchial and cardiac, may also share a "final common pathway" in their development, i.e., reflex contraction of the airway smooth muscle. In bronchial asthma, it is thought that the condition may be brought about by a reflex bronchoconstriction in susceptible subjects (217) due to inhaled allergens setting up an allergic reaction leading to the release of mediators such as histamine and prostaglandins which in turn activate sensory endings in the epithelium of the airways, besides having a direct effect on the airway smooth muscle (84,215,

232). The afferents involved are thought to be RAR, pulmonary and bronchial C-fibre receptors. In cardiac asthma, the presence of a reflex bronchoconstriction as suggested by the findings of this study may play a role in the development of "bronchospasm". The relative importance of this mechanism, as compared to the other suggested mechanisms involving the direct effects of the oedema fluid in causing changes in lung mechanisms (2,3,4), is not known. However, the presence of this reflex bronchoconstriction may alter the strategy in the management of cardiac asthma.

Strategy for Management

The principal aims in the management of cardiac asthma are (a) removing or changing the precipitating cause, e.g., impaired myocardial contractility or malfunctioning cardaic valves, and (b) removing the excessive fluid build-up in the lungs. However, means to achieve these aims may not be available immediately, and more importantly, the desired therapeutic results may not be achieved quickly or readily. For example, the impaired left ventricular function following myocardial infarction may not readily or may only partially respond to positive inotropic and afterload reduction therapy. In other cases, the malfunctioning valve (stenotic or incompetent) may only be effectively managed by valve replacement surgery. Also, time is needed for the diuretic therapy to remove the excessive fluid build-up. In the meantime, the respiratory distress of cardiac asthma may contribute to the vicious cycle of deteriorating cardiac function because of (a) the increased oxygen demand by the over-worked respiratory muscles, and (b) the associated hypoxia and acidosis. Thus, relieving the reflex bronchoconstriction

will reduce the work of breathing and improve the delivery of oxygen to the alveoli for gaseous exchange. This may help alleviate the problems associated with cardiac asthma. If this approach is effective immediately, then it will augment the main therapeutic measures taken to deal with the pulmonary oedema.

There is no evidence that such an approach will be effective. But, perhaps the use of a theophylline compound, aminophylline, during cardiac asthma is based on this rationale though this compound is nowadays rarely used for such a purpose.

The results of this study suggest that the reflex bronchoconstriction is mainly mediated by the vagal parasympathetic nervous system and not from lack of beta$_2$-adrenergic stimulation. Perhaps, the search should be directed towards drugs that are atropinic. However, bearing in mind that during an attack of cardiac asthma, the heart is already under a heightened sympathetic influence, such drugs would have to be as non-arrhythmogenic as possible. Thus, just as there is a search for the ideal atropinic drug to relieve the bronchoconstriction of bronchial asthma, perhaps a search should be initiated for a similar drug for cardiac asthma.

CONCLUSION

This study investigated the responses of the pulmonary receptors to pulmonary venous congestion produced by partial obstruction of the mitral valve. The results showed that these receptors demonstrated a differential response to the stimulus which was a relatively mild degree of pulmonary congestion. The RAR showed the most prominant response when so stimulated.

It was found that this form of pulmonary venous congestion produced a five fold increase in pulmonary lymphatic flow and also caused a small but significant decrease in dynamic compliance of the lungs.

Finally, it was demonstrated that a reflex contraction of an upper tracheal segment occurred in response to the pulmonary venous congestion. It was established that the most likely afferent limb of the reflex was formed by the the RAR endings in the airways and lungs and discharging into afferent fibres in the vagi. The efferent limb was via the parasympathetic nerve fibres in the superior laryngeal nerves to the posterior trachealis muscle.

An alternative hypothesis for the activation of the RAR has been suggested. A possible mechanism in the development of the respiratory wheeze during cardiac asthma has been presented. However, these suggestions raise a number of questions that need to be answered.

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